TECHNICAL MANUAL

PowerPlex® 16 System

Instructions for Use of Products
DC6530 and DC6531

Revised 5/16
TMD012

PowerPlex® 16 System

Promega

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 (5–8). 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® 16 System[a–d] (9,10) is used for human identification applications including forensic analysis, relationship testing and research use. The system allows co-amplification and three-color detection of sixteen loci (fifteen STR loci and Amelogenin), including Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818. One primer for each of the Penta E, D18S51, D21S11, TH01 and D3S1358 loci is labeled with fluorescein (FL); one primer for each of the FGA, TPOX, D8S1179, vWA and Amelogenin loci is labeled with carboxy-tetramethylrhodamine (TMR); and one primer for each of the Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818 loci is labeled with 6-carboxy-4´,5´-dichloro-2´,7´-dimethoxy-fluorescein (JOE). All sixteen loci are amplified simultaneously in a single tube and analyzed in a single injection.

The PowerPlex® 16 Monoplex System, Penta E (Fluorescein) (Cat.# DC6591) and PowerPlex® 16 Monoplex System, Penta D (JOE) (Cat.# DC6651) are available to amplify the Penta E and Penta D loci, respectively. Each monoplex system allows amplification of a single locus to confirm results obtained with the PowerPlex® 16 System. The monoplex systems also can be used to re-amplify DNA samples when one or more of the loci do not amplify initially due to nonoptimal amplification conditions or poor DNA template quality.

The PowerPlex® 16 System is compatible with the ABI PRISM® 310, 3100 and 3100-*Avan*t Genetic Analyzers and Applied Biosystems® 3130 and 3130*xl* Genetic Analyzers. The protocols presented in this manual were tested at Promega Corporation. Amplification and detection instrumentation may vary. You may need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. In-house validation should be performed.

The PowerPlex® 16 System provides all materials necessary to amplify STR regions of human genomic DNA except for AmpliTaq Gold® DNA polymerase. This manual contains protocols for use of the PowerPlex® 16 System with the Perkin-Elmer model 480 and GeneAmp® PCR System 9600, 9700 and 2400 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**

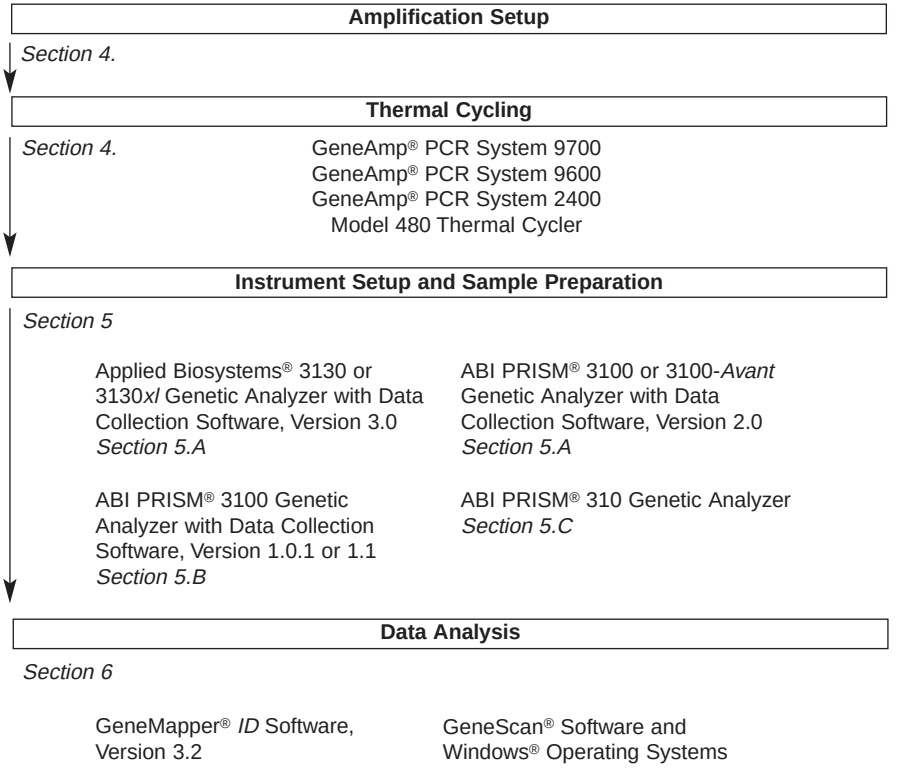

Figure 1. An overview of the PowerPlex® 16 System protocol.

2. Product Components and Storage Conditions

Product	Size	Cat.#
PowerPlex® 16 System	100 reactions	DC6531

Not For Medical Diagnostic Use. Cat.# DC6531 contains sufficient reagents for 100 reactions of 25µl each. Includes:

Pre-amplification Components Box

1 × 300µl	Gold ST★R 10X Buffer
1 × 250µl	PowerPlex® 16 10X Primer Pair Mix
25µl	2800M Control DNA, 10ng/µl

Post-amplification Components Box

1 × 50µl	PowerPlex® 16 Allelic Ladder Mix
1× 150µl	Internal Lane Standard (ILS) 600

Product	Size	Cat.#
PowerPlex® 16 System	400 reactions	DC6530

Not For Medical Diagnostic Use. Cat.# DC6530 contains sufficient reagents for 400 reactions of 25µl each. Includes:

Pre-amplification Components Box

4 × 300µl	Gold ST★R 10X Buffer
4 × 250µl	PowerPlex® 16 10X Primer Pair Mix
25µl	2800M Control DNA, 10ng/µl

Post-amplification Components Box

4 × 50µl	PowerPlex® 16 Allelic Ladder Mix
4 × 150µl	Internal Lane Standard (ILS) 600

The PowerPlex® 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.

Storage Conditions: 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. The PowerPlex® 16 10X Primer Pair Mix, PowerPlex® 16 Allelic Ladder Mix and Internal Lane Standard 600 are light-sensitive and must be stored in the dark. We strongly recommend that pre-amplification and post-amplification reagents be stored and used separately with different pipettes, tube racks, etc.

The PowerTyper™ Macros (Release 2.0) for use with Genotyper® software can be downloaded at:
www.promega.com/resources/tools/powertyper-macros/

The proper panels and bins text files for use with GeneMapper® *ID* 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 sold separately and are available for the ABI PRISM® 310 Genetic Analyzer (PowerPlex® Matrix Standards, 310) and ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130 and 3130*xl* Genetic Analyzers (PowerPlex® 4C Matrix Standard). See Section 9.F for ordering information.

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 (11,12). Guidelines for the validation process are published in the *Internal Validation Guide of Autosomal STR Systems for Forensic Laboratories* (13).

The quality of purified DNA or direct-amplification samples, small changes in buffers, ionic strength, primer concentrations, choice of thermal cycler and thermal cycling conditions can affect PCR success. We suggest strict adherence to recommended procedures for amplification and fluorescence detection. Additional research and validation are required if any modifications to the recommended protocols are made.

PCR-based STR analysis is subject to contamination by very small amounts of human DNA. Extreme care should be taken to avoid cross-contamination when preparing template DNA, handling primer pairs, assembling amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (Gold ST★R 10X Buffer and PowerPlex® 16 10X Primer Pair Mix) are provided in a separate box and should be stored separately from those used following amplification (PowerPlex® 16 Allelic Ladder Mix and Internal Lane Standard 600). Always include a negative control reaction (i.e., no template) to detect reagent contamination. We highly recommend the use of gloves and aerosol-resistant pipette tips.

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 and 3130*xl* Genetic Analyzers. A matrix must be generated for each individual instrument.

The PowerPlex® Matrix Standards, 310 (Cat.# DG4640), is required for matrix standardization for the ABI PRISM® 310 Genetic Analyzer. The PowerPlex® 4C Matrix Standard (Cat.# DG4800) cannot be used to generate a matrix on the ABI PRISM® 310 Genetic Analyzer.

The PowerPlex® 4C Matrix Standard is required for spectral calibration on the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130 and 3130*xl* Genetic Analyzers. The PowerPlex® Matrix Standards, 310, cannot be used to generate a matrix on these instruments.

For protocols and additional information about matrix generation and spectral calibration, see the *PowerPlex® Matrix Standards, 310, Technical Bulletin* #TBD021. For protocols and additional information about spectral calibration, see the *PowerPlex® 4C Matrix Standard, Technical Bulletin* #TMD048. These manuals are available online at: **www.promega.com/protocols/**

4. Protocols for DNA Amplification Using the PowerPlex® 16 System

The PowerPlex® 16 System is optimized for the GeneAmp® PCR System 9700 thermal cycler. Amplification protocols for the GeneAmp® PCR Systems 9600 and 2400 thermal cyclers and Perkin-Elmer model 480 thermal cycler are provided.

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.

4.A. Amplification of Extracted DNA

Materials to Be Supplied by the User

- model 480 or GeneAmp® PCR System 9600, 9700 or 2400 thermal cycler (Applied Biosystems)
- microcentrifuge
- MicroAmp® optical 96-well reaction plate or 0.5ml GeneAmp® or 0.2ml MicroAmp® reaction tubes (Applied Biosystems)
- aerosol-resistant pipette tips
- AmpliTaq Gold® DNA polymerase (Applied Biosystems)
- Nuclease-Free Water (Cat.# P1193)
- Mineral Oil (for use with the model 480 thermal cycler)

We routinely amplify 0.5–1ng of template DNA in a 25µl reaction volume using the protocols detailed below. Developmental validation of the kit showed routine generation of full profiles with lower amounts of DNA template down to 125pg (9). Partial profiles were typically observed for DNA template of 62pg and below. Expect to see higher peak heights at the smaller loci and lower peak heights at the larger loci if more than the recommended amount of template is used. Reduce the amount of template DNA or number of cycles to correct this. We recommend that you perform optimization and validation of the kit to establish its performance in your laboratory.

Store DNA to be used for sensitivity studies at 4°C overnight before use.

Amplification Setup

1. Thaw the Gold ST★R 10X Buffer and PowerPlex® 16 10X Primer Pair Mix completely.

   Notes:

   1. 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 after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.
   2. A precipitate may form in the Gold ST★R 10X Buffer. If this occurs, warm the solution briefly at 37°C, then vortex until the precipitate is in solution.

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.

   Note: If using the GeneAmp® PCR System 9600, 9700 or 2400 thermal cyclers, use a MicroAmp® plate or 0.2ml MicroAmp® 8-strip reaction tubes. For the model 480 thermal cycler, we recommend 0.5ml GeneAmp® thin-walled reaction tubes.

4.A. Amplification of Extracted DNA (continued)

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

Amplification of >1ng of DNA template results in an imbalance in peak heights from locus to locus. The smaller loci show greater amplification yield than the larger loci. Reducing the number of cycles in the amplification program by 2 to 4 cycles (i.e., 10/20 or 10/18 cycling) can improve locus-to-locus balance.

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

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume
nuclease-free water	to a final volume of 25.0µl	×		=	
Gold ST★R 10X Buffer	2.5µl	×		=	
PowerPlex® 16 10X Primer Pair Mix	2.5µl	×		=	
AmpliTaq Gold® DNA polymerase[2]	0.8µl (4u)	×		=	
template DNA (0.5–1.0ng)[3,4,5]	up to 19.2µl				
total reaction volume	25µl				

[1]Add nuclease-free water to the tube first, then add Gold ST★R 10X Buffer, PowerPlex® 16 10X Primer Pair Mix and AmpliTaq Gold® DNA polymerase. The template DNA will be added at Step 6.

[2]Assumes the AmpliTaq Gold® DNA polymerase is at 5u/µl. If the enzyme concentration is different, the volume of enzyme must be adjusted accordingly.

[3]Store DNA templates in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or TE^{-4} buffer with 20µg/ml glycogen. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the volume of DNA added should not exceed 20% of the final reaction volume. 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.

[4]Apparent DNA concentrations can differ, depending on the DNA quantification method used (14). The amount of DNA template recommended here is based on DNA concentrations determined by measuring absorbance at 260nm. We strongly recommend that you perform experiments to determine the optimal DNA amount based on your DNA quantification method.

[5]The PowerPlex® 16 System is optimized and balanced for 0.5–1.0ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your internal validation and may be different.

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

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

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

   Note: The PowerPlex® 16 System is optimized and balanced for 0.5–1.0ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your internal validation and may be different.

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

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

9. If using the model 480 thermal cycler and GeneAmp® reaction tubes, add one drop of mineral oil to each tube before closing. If using the GeneAmp® PCR System 9600, 9700 or 2400 thermal cycler and MicroAmp® reaction tubes or plates, no addition of mineral oil to the reaction wells or tubes is required.

   Note: Allow the mineral oil to flow down the side of the tube and form an overlay to limit sample loss or cross-contamination due to splattering.

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

Thermal Cycling

This section contains protocols for use of the PowerPlex® 16 System with the model 480 and GeneAmp® PCR system 9600, 9700 and 2400 thermal cyclers. For information about other thermal cyclers, contact Promega Technical Services by e-mail: **genetic@promega.com**

Amplification and detection instrumentation may vary. You may need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 10/22 cycles work well for 0.5–1ng of purified DNA templates. For higher template amounts or to decrease sensitivity, fewer cycles, such as 10/16, 10/18 or 10/20, should be evaluated. 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 protocols for use with the GeneAmp® PCR System 9600, 9700 and 2400 thermal cyclers and model 480 thermal cycler are 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.

Protocol for the GeneAmp® PCR System 9700 Thermal Cycler[1]	Protocol for the GeneAmp® PCR System 2400 Thermal Cycler
95°C for 11 minutes, then:	95°C for 11 minutes, then:
96°C for 1 minute, then:	96°C for 1 minute, then:
ramp 100% to 94°C for 30 seconds ramp 29% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for 10 cycles, then:	ramp 100% to 94°C for 30 seconds ramp 100% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for 10 cycles, then:
ramp 100% to 90°C for 30 seconds ramp 29% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for 22 cycles, then:	ramp 100% to 90°C for 30 seconds ramp 100% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for 22 cycles, then:
60°C for 30 minutes	60°C for 30 minutes
4°C soak	4°C soak
Protocol for the GeneAmp® PCR System 9600 Thermal Cycler	**Protocol for the Model 480 Thermal Cycler**
95°C for 11 minutes, then:	95°C for 11 minutes, then:
96°C for 1 minute, then:	96°C for 2 minutes, then:
94°C for 30 seconds ramp 68 seconds to 60°C (hold for 30 seconds) ramp 50 seconds to 70°C (hold for 45 seconds) for 10 cycles, then:	94°C for 1 minute 60°C for 1 minute 70°C for 1.5 minutes for 10 cycles, then:
90°C for 30 seconds ramp 60 seconds to 60°C (hold for 30 seconds) ramp 50 seconds to 70°C (hold for 45 seconds) for 22 cycles, then:	90°C for 1 minute 60°C for 1 minute 70°C for 1.5 minutes for 22 cycles, then:
60°C for 30 minutes	60°C for 30 minutes
4°C soak	4°C soak

[1]When using the GeneAmp® PCR System 9700 thermal cycler, the ramp rates indicated in the cycling program must be set, and the program must be run in 9600 ramp mode.

The ramp rates are set in the Ramp Rate Modification screen. While viewing the cycling program, navigate to the Ramp Rate Modification screen by selecting "More", then "Modify". On the Ramp Rate Modification screen the default rates for each step are 100%. The rate under each hold step is the rate at which the temperature will change to that hold temperature. Figure 2 shows the ramp rates for the GeneAmp® PCR System 9700 thermal cycler.

The ramp mode is set after "start" has been selected for the thermal cycling run. A Select Method Options screen appears. Select 9600 ramp mode, and enter the reaction volume.

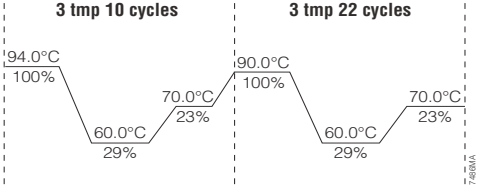

Figure 2. The ramp rates for the GeneAmp® PCR System 9700 thermal cycler.

4.B. Direct Amplification of DNA from nonFTA 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
- AmpliTaq Gold® DNA polymerase (Applied Biosystems)
- Nuclease-Free Water (Cat.# P1193)
- PunchSolution™ Kit (Cat.# DC9271)
- 5X AmpSolution™ Reagent (Cat.# DM1231), also supplied with the PunchSolution™ Kit
- 1.2mm Harris Micro-Punch or equivalent manual punch and cutting mat

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

When using the protocol detailed below, add one 1.2mm storage card punch to each 25µl amplification reaction.

NonFTA sample types include:

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

We do not recommend amplification of DNA from blood samples on nonFTA cards using the PowerPlex® 16 System.

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

Use a manual punch tool with a 1.2mm tip to manually create sample disks from a storage card. Place tip near the center of the sample spot, and with a twisting or pressing action, cut a 1.2mm sample disk. Use the plunger to eject the disk into the appropriate well of a reaction plate.

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

Note: Static may be problematic when adding a punch to a well. Adding PunchSolution™ Reagent to the well before adding the punch during pretreatment may help alleviate static problems.

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

Amplification Setup

1. Thaw the Gold ST★R 10X Buffer and PowerPlex® 16 10X Primer Pair Mix completely.

   Notes:

   1. 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 after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.
   2. A precipitate may form in the Gold ST★R 10X Buffer. If this occurs, warm the solution briefly at 37°C, then vortex until the precipitate is in solution.

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 to 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
nuclease-free water	14.2µl	×		=	
Gold ST★R 10X Buffer	2.5µl	×		=	
PowerPlex® 16 10X Primer Pair Mix	2.5µl	×		=	
AmpliTaq Gold® DNA polymerase[2]	0.8µl (4u)	×		=	
5X AmpSolution™ Reagent	5.0µl	×		=	
total reaction volume	25µl				

[1]Add nuclease-free water to the tube first, then add Gold ST★R 10X Buffer, PowerPlex®16 10X Primer Pair Mix, AmpliTaq Gold® DNA polymerase and 5X AmpSolution™ Reagent. The template DNA will be added at Step 6.

5. Vortex the PCR amplification mix for 5–10 seconds.

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

6. Pipet 25µl of PCR amplification mix into each reaction well with one pretreated 1.2mm punch from a nonFTA storage card containing a buccal sample. Pipet 25µl of PCR amplification mix into each reaction well for the control reactions.

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

   Notes:

   1. Do not include a blank storage card punch in the positive control reactions.
   2. Optimization of the amount of 2800M Control DNA may be required depending on thermal cycling conditions and laboratory preferences. Typically, 10ng of 2800M Control DNA is sufficient to provide a robust profile using the cycle numbers recommended here. A one-cycle reduction in cycle number will require a twofold increase in mass of DNA template to generate similar signal intensity. Similarly, a one-cycle increase in cycle number will require a twofold reduction in the amount of 2800M Control DNA to avoid signal saturation.

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.

9. Seal the plate, and briefly centrifuge the plate to bring the storage card punch 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 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).

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

Thermal Cycling

Amplification and detection instrumentation may vary. You will need to optimize protocols including cycle number, injection conditions and loading volume for each laboratory instrument. Testing at Promega shows that 27 cycles works well for a variety of nonFTA sample types. 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 Protocol[1]
95°C for 11 minutes, then:
96°C for 1 minute, then:
ramp 100% to 94°C for 30 seconds ramp 29% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for 10 cycles, then:
ramp 100% to 90°C for 30 seconds ramp 29% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for 17 cycles, then:
60°C for 30 minutes
4°C soak

[1]When using the GeneAmp® PCR System 9700 thermal cycler, the ramp rates indicated in the cycling program must be set, and the program must be run in 9600 ramp mode. The ramp rates are set in the Ramp Rate Modification screen. While viewing the cycling program, navigate to the Ramp Rate Modification screen by selecting "More", then "Modify". On the Ramp Rate Modification screen the default rates for each step are 100%. The rate under each hold step is the rate at which the temperature will change to that hold temperature. Figure 2 shows the ramp rates for the GeneAmp® PCR System 9700 thermal cycler.

The ramp mode is set after "start" has been selected for the thermal cycling run. A Select Method Options screen appears. Select 9600 ramp mode, and enter the reaction volume.

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

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

PCR Optimization

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

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.
2. Place one 1.2mm nonFTA storage card punch in each well of a reaction plate. Be sure to pretreat nonFTA samples with the PunchSolution™ Kit (Cat.# DC9271).
3. Prepare three identical reaction plates with punches from the same samples.
4. Amplify samples using the thermal cycling protocol provided above, but subject each plate to a different cycle number.
5. Following amplification, use your laboratory's validated separation and detection protocols to determine the optimal cycle number for the sample type.

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
- AmpliTaq Gold® DNA polymerase (Applied Biosystems)
- Nuclease-Free Water (Cat.# P1193)
- SwabSolution™ Kit (Cat.# DC8271)
- 5X AmpSolution™ Reagent (Cat.# DM1231), also supplied with the SwabSolution™ Kit

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

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

Amplification Setup

1. Thaw the Gold ST★R 10X Buffer and PowerPlex® 16 10X Primer Pair Mix completely.

   Notes:

   1. 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 after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.
   2. A precipitate may form in the Gold ST★R 10X Buffer. If this occurs, warm the solution briefly at 37°C, then vortex until the precipitate is in solution.

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

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 to a sterile tube.

Table 3. 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
nuclease-free water	12.2µl	×		=	
Gold ST★R 10X Buffer	2.5µl	×		=	
PowerPlex® 16 10X Primer Pair Mix	2.5µl	×		=	
AmpliTaq Gold® DNA polymerase[2]	0.8µl (4u)	×		=	
5X AmpSolution™ Reagent	5.0µl	×		=	
swab extract	2µl				
total reaction volume	25µl				

[1]Add nuclease-free water to the tube first, then add Gold ST★R 10X Buffer, PowerPlex®16 10X Primer Pair Mix, AmpliTaq Gold® DNA polymerase 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 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, vortex the tube of 2800M Control DNA, then dilute to 2.5ng/µl. Add 2µl (5ng) to a reaction well containing 23µl of PCR amplification mix.

   Note: Optimization of the amount of 2800M Control DNA may be required depending on thermal cycling conditions and laboratory preferences.
8. For the negative amplification control, pipet 2µl of Water, Amplification Grade, or TE^{-4} buffer instead of swab extract into a reaction well containing PCR amplification mix.

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

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 the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 28 cycles works well for a variety of sample types. Cycle number will need to be optimized in each laboratory for each sample type that is amplified (see below).

1. Place the 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 Protocol[1]
95°C for 11 minutes, then:
96°C for 1 minute, then:
ramp 100% to 94°C for 30 seconds ramp 29% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for 10 cycles, then:
ramp 100% to 90°C for 30 seconds ramp 29% to 60°C for 30 seconds ramp 23% to 70°C for 45 seconds for 18 cycles, then:
60°C for 30 minutes
4°C soak

[1]When using the GeneAmp® PCR System 9700 thermal cycler, the ramp rates indicated in the cycling program must be set, and the program must be run in 9600 ramp mode. The ramp rates are set in the Ramp Rate Modification screen. While viewing the cycling program, navigate to the Ramp Rate Modification screen by selecting "More", then "Modify". On the Ramp Rate Modification screen the default rates for each step are 100%. The rate under each hold step is the rate at which the temperature will change to that hold temperature. Figure 2 shows the ramp rates for the GeneAmp® PCR System 9700 thermal cycler.

The ramp mode is set after "start" has been selected for the thermal cycling run. A Select Method Options screen appears. Select 9600 ramp mode, and enter the reaction volume.

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

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

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 (27, 28 and 29 cycles).

   Note: This recommendation is for 2µl of swab extract.
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 ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, or the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3100 or 3130 capillary array, 36cm
- performance optimized polymer 4 (POP-4® polymer) for the 3100 or 3130
- 10X genetic analyzer buffer with EDTA
- 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. Thaw the Internal Lane Standard 600.

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

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

   [(0.5µl ILS 600) × (# samples)] + [(9.5µl Hi-Di™ formamide) × (# samples)]

   Note: The volume of internal lane standard used in the loading cocktail can be increased or decreased to adjust the intensity of the size standard peaks. The optimal peak height for the 100-base fragment of the internal lane standard is 500–1,000RFU. If peak heights are too low, we recommend altering the formamide/internal lane standard mix to contain 1.0µl of ILS 600 and 9.0µl of Hi-Di™ formamide. If peak heights are too high, we recommend altering the loading cocktail to contain 0.25µl of ILS 600 and 9.75µl of formamide.

3. Vortex for 10–15 seconds to mix.

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

5. Add 1µl of amplified sample (or 1µl of PowerPlex® 16 Allelic Ladder Mix). Cover wells with appropriate septa.

   Note: Instrument detection limits vary; therefore, injection time, injection voltage or the amount of product mixed with loading cocktail may need to be adjusted. Use the Module Manager in the data collection software to modify the injection time or voltage in the run module. If peak heights are higher than desired, samples can be diluted in Gold ST★R 1X Buffer before mixing with loading cocktail. This may result in uneven allele peak heights across loci. For best results, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program by 2–4 cycles to achieve the desired signal intensity.

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

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

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

Instrument Preparation

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

Analyze samples as described in the user's manual for the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, and the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0, with the following exceptions.

1. In the Module Manager, select "New". Select "Regular" in the Type drop-down list, and select "HIDFragmentAnalysis36_POP4" in the Template drop-down list. Confirm that the injection time is 5 seconds and the injection voltage is 3kV. Lengthen the run time to 2,000 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 "F" in the Dye-Set drop-down list. Select "OK.

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

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

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

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

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

6. In the spectral viewer, confirm that dye set F is active, and set the correct active calibration for dye set F.

7. In the run scheduler, locate the plate record that you just created in Steps 3 and 4, and click once on the name to highlight it.
8. Once the plate record is highlighted, click the plate graphic that corresponds to the plate on the autosampler that contains your amplified samples.
9. When the plate record is linked to the plate, the plate graphic will change from yellow to green, and the green Run Instrument arrow becomes enabled.
10. Click on the green Run Instrument arrow on the toolbar to start the sample run.
11. Monitor electrophoresis by observing the run, view, array or capillaries viewer window in the data collection software. Each injection will take approximately 45 minutes.

5.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 Genetic Analyzer with Data Collection Software, Version 1.0.1 or 1.1

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 capillary array, 36cm
- performance optimized polymer 4 (POP-4® polymer) for the 3100
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate (or equivalent) and septa for the 3100
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

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

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

Sample Preparation

1. Thaw the Internal Lane Standard 600.

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

5.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 Genetic Analyzer with Data Collection Software, Version 1.0.1 or 1.1 (continued)

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

   [(0.5µl ILS 600) × (# samples)] + [(9.5µl Hi-Di™ formamide) × (# samples)]

   Note: The volume of internal lane standard used in the loading cocktail can be increased or decreased to adjust the intensity of the size standard peaks. If peak heights are too low, we recommend altering the formamide/internal lane standard mix to contain 1.0µl of ILS 600 and 9.0µl of Hi-Di™ formamide. If peak heights are too high, we recommend altering the loading cocktail to contain 0.25µl of ILS 600 and 9.75µl of formamide.

3. Vortex for 10–15 seconds to mix.

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

5. Add 1µl of amplified sample (or 1µl of PowerPlex® 16 Allelic Ladder Mix) to each well. Cover wells with appropriate septa.

   Note: Instrument detection limits vary; therefore, injection time, injection voltage or the amount of product mixed with loading cocktail may need to be increased or decreased. Use the Module Editor in the data collection software to modify injection time or voltage in the run module. If peak heights are higher than desired, samples can be diluted in Gold ST★R 1X Buffer before mixing with loading cocktail. The use of too much template DNA may result in uneven allele peak heights across loci. For best results, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program to achieve the desired signal intensity.

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

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

Instrument Preparation

Refer to the *ABI PRISM® 3100 Genetic Analyzer User's Manual* for instructions on cleaning the blocks, installing the capillary array, performing a spatial calibration and adding polymer to the reserve syringe.

1. Open the ABI PRISM® 3100 Data Collection Software.

2. Change the "GeneScan36_POP4DefaultModule" module run time to 2,000 seconds.

3. Change the injection voltage to 3kV.

4. Change the injection time to 11 seconds.

   Note: Instrument sensitivities can vary. 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.

5. Save the module with a new name (e.g., GeneScan36_POP4PowerPlex16_3kV_11secs_2000). Use this as the initial run module for all runs.

6. Open a new plate record. Name the plate, and select "GeneScan". Select the plate size (96-well). Select "Finish".

7. Complete the plate record spreadsheet for the wells you have loaded. Enter appropriate information into the Sample Name and Color Info columns. For allelic ladder samples, insert the word "ladder" into the Color Info column for the blue, yellow and green dye colors. This information must be entered to successfully analyze data with the PowerTyper™ 16 Macro (Release 2.0).

8. In the BioLIMS Project column, select "3100_Project1" from the drop-down menu.

9. In the Dye Set column, select "Z" from the drop-down menu.

10. When using the ABI PRISM® 3100 Data Collection Software, Version 1.0.1 or 1.1, select "GeneScan36_POP4PowerPlex16_3kV_11secs_2000" from the drop-down menu in the Run Module 1 column.

11. To collect the data without autoanalyzing, select "No Selection" in the Analysis Module 1 column. Analysis parameters can be applied after data collection and during data analysis using the GeneScan® software.

12. Select "OK". This new plate record will appear in the pending plate records table on the plate setup page of the collection software.

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

14. Locate the pending plate record that you just created, and click once on the name.

15. Once the pending plate record is highlighted, click on the plate graphic that corresponds to the plate on the autosampler that contains your amplified samples to link the plate to the plate record.

16. When the plate record is linked to the plate, the plate graphic will change from yellow to green, the plate record moves from the pending plate records table to the linked plate records table, and the Run Instrument button becomes enabled.

17. Select "Run Instrument" on the toolbar to start the sample run.

18. Monitor electrophoresis by observing the run, status, array and capillary views windows in the collection software. Each injection will take approximately 45 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
- 310 capillaries, 47cm × 50µm
- performance optimized polymer 4 (POP-4® polymer)
- 10X genetic analyzer buffer with EDTA
- sample tubes and septa
- aerosol-resistant pipette tips
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)
- crushed ice or ice-water bath

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

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

Sample Preparation

1. Thaw the Internal Lane Standard 600.

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

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

   [(1.0µl ILS 600) × (# samples)] + [(24.0µl Hi-Di™ formamide) × (# samples)]

   Note: The volume of internal lane standard used in the loading cocktail can be increased or decreased to adjust the intensity of the size standard peaks. If peak heights are too high, we recommend altering the loading cocktail to contain 0.5µl of ILS 600 and 24.5µl of Hi-Di™ formamide.

3. Vortex for 10–15 seconds to mix.

4. Combine 25.0µl of prepared loading cocktail and 1.0µl of amplified sample (or 1µl of PowerPlex® 16 Allelic Ladder Mix).

   Note: Instrument detection limits vary; therefore, injection time, injection voltage or the amount of product mixed with loading cocktail may need to be increased or decreased. If peak heights are higher than desired, samples can be diluted in Gold ST★R 1X Buffer before mixing with loading cocktail. This may result in uneven allele peak heights across loci. For best results, use less template DNA in the amplification reactions or reduce the number of cycles in the amplification program by 2–4 cycles (e.g. 10/18 or 10/20 cycling).

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

6. Denature samples 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.
7. Assemble tubes in the appropriate autosampler tray.
8. Place the autosampler tray in the instrument, and close the instrument doors.

Instrument Preparation

Refer to the instrument users' 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.

   For rows containing PowerPlex® 16 Allelic Ladder Mix, insert the word "ladder" in the Sample Info column for the blue dye color, yellow dye color and green dye color. This information must be entered to successfully analyze your data using the PowerTyper™ 16 Macro (Release 2.0).
3. Create a new GeneScan® injection list. Select the appropriate sample sheet from the drop-down menu.
4. Select the "GS STR POP4 (1ml) F" Module using the drop-down menu. Change the injection time to 3 seconds and the run time to 30 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:	30

   You may need to optimize the injection time for individual instruments. Injection times of 2–5 seconds are suggested for samples that contain 1ng 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 (Section 3.B).
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 on these options.

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

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 40 minutes for syringe pumping, sample injection and sample electrophoresis.

6. Data Analysis

6.A. Importing PowerPlex® Panels and Bins Text Files into GeneMapper® *ID*, Version 3.2

To facilitate analysis of data generated with the PowerPlex® 16 System, we have created panels and bins text files to allow automatic assignment of genotypes using GeneMapper® *ID* software, version 3.2. We recommend that users of GeneMapper® *ID* software, version 3.2, complete the *Applied Biosystems GeneMapper®* ID *Software Human Identification Analysis Tutorial* to familiarize themselves with proper operation of the software. For GeneMapper® *ID* software, version 3.1, users we recommend upgrading to version 3.2.

Getting Started

1. To obtain the panels and bins text files for use with the PowerPlex®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 Promega_Panels_ID3.2.X.txt and Promega_Bins_ID3.2.X.txt files, where "X" refers to the most recent version of the panels and bins text files, 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 text file downloaded in the Getting Started section above. Select the file, then "Import".

6. In the navigation pane, highlight the Promega Panels ID3.2.X folder that you just imported in Step 5.
7. Select "File", then "Import Bin Set".
8. Navigate to the bins text file downloaded in the Getting Started section above. Select the file, then "Import".
9. At the bottom of the Panel Manager window, select "OK". This will save the panels and bins text files and close the window.

6.B. Creating a Size Standard with GeneMapper® *ID* Software, Version 3.2

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

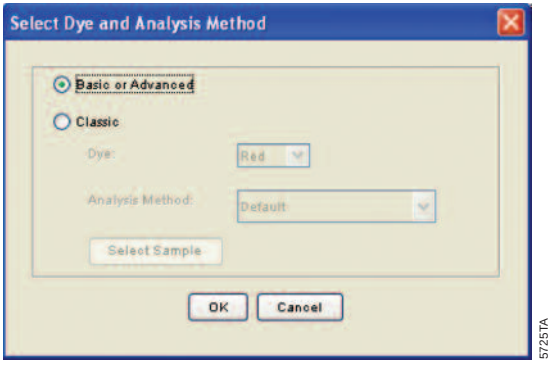

Figure 3. The Select Dye and Analysis Method window.

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

5. Enter a detailed name, such as "ILS 600 Advanced", in the Size Standard Editor (Figure 4).

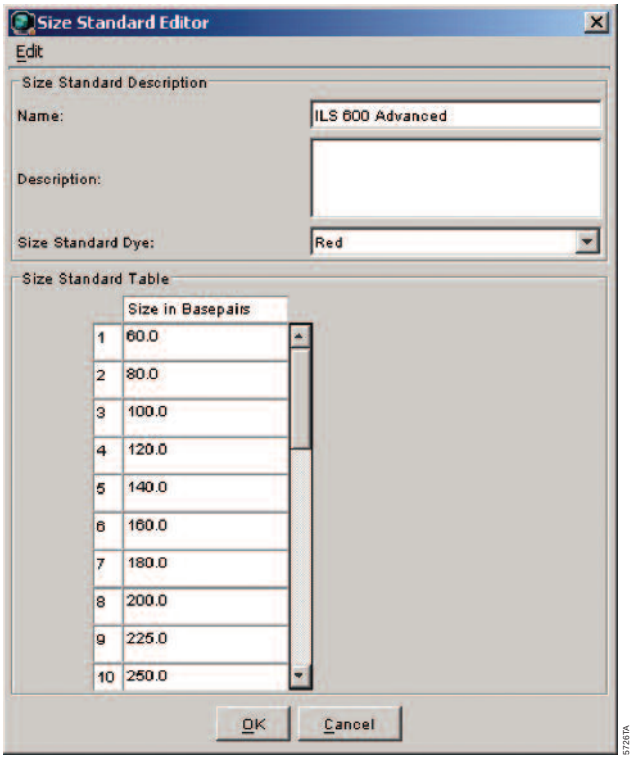

Figure 4. The Size Standard Editor.

6. Choose "Red" for the Size Standard Dye.
7. Enter the sizes of the internal lane standard fragments (60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases). See Section 9.D, Figure 13.
8. Select "OK".

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

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

1. Select "Tools", then "GeneMapper Manager".
2. Select the Analysis Methods tab.

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 "PowerPlex16 advanced".
6. Select the Allele tab (Figure 5).
7. Select the bins text file that was imported in Section 6.A.
8. Ensure that the "Use marker-specific stutter ratio if available" box is checked.
9. Enter the values shown in Figure 5 for proper filtering of stutter peaks when using the PowerPlex® 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.

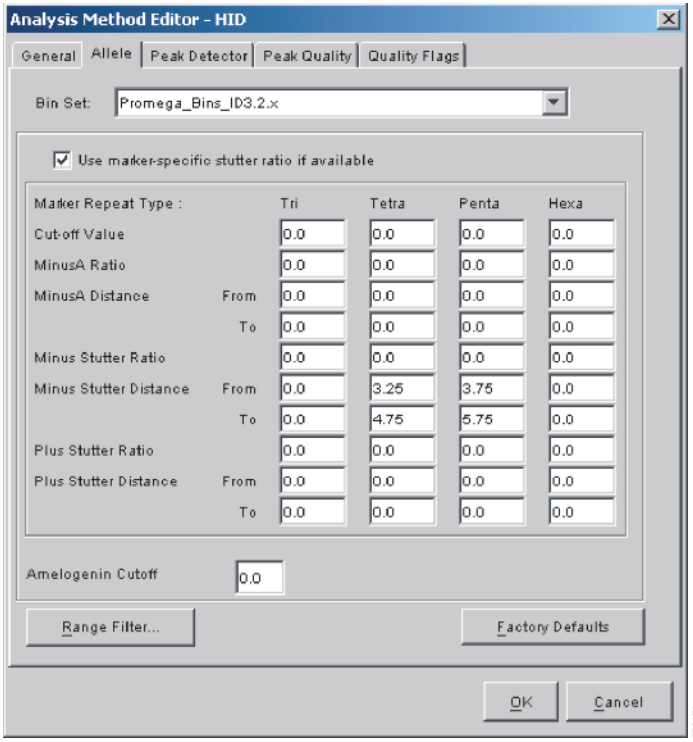

Figure 5. The Allele tab.

10. Select the Peak Detector tab. We recommend the settings shown in Figure 6.

    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.

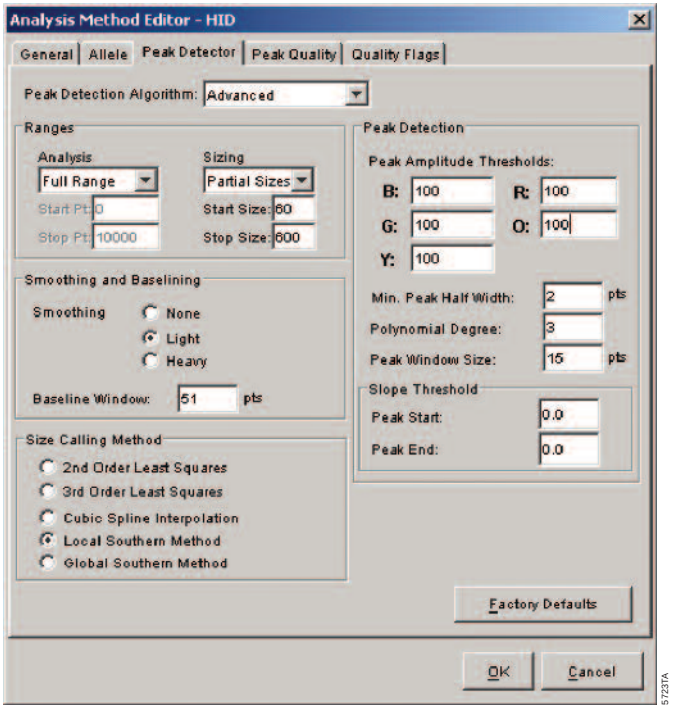

Figure 6. The Peak Detector tab.

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

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

13. Select "OK" to save your settings.

Processing Data for Casework Samples

1. Select "File", then "New Project".
2. Select "Edit", then "Add Samples to Project".
3. Browse to the location of the run files. Highlight desired files, then select "Add to list" followed by "Add".
4. In the Sample Type column, use the drop-down menu to select "Ladder", "Sample", "Positive Control" or "Negative Control" as appropriate for the sample. Every folder in the project must contain at least one allelic ladder that is designated as such for proper genotyping.
5. In the Analysis Method column, select the analysis method created previously in this section.
6. In the Panel column, select the panels text file that was imported in Section 6.A.
7. In the Size Standard column, select the size standard that was created in Section 6.B.
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.D. 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 "PowerPlex16_20%filter".
6. Select the Allele tab (Figure 7).
7. Select the bins text file that was imported in Section 6.A.
8. Ensure that the "Use marker-specific stutter ratio if available" box is checked.

9. Enter the values shown in Figure 7 for proper filtering of peaks when using the PowerPlex® 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".

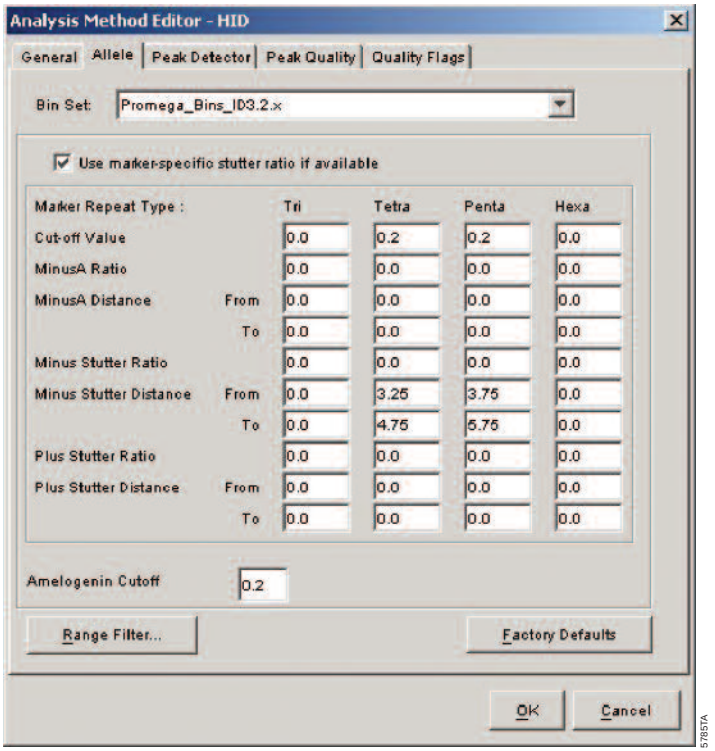

Figure 7. The Allele tab with settings for using a 20% peak filter.

10. Select the Peak Detector tab. We recommend the settings shown in Figure 6.

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 the location of the run files. Highlight desired files, then select "Add to list" followed by "Add".

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

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

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

7. In the Size Standard column, select the size standard that was created in Section 6.B.

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.E. Sample Analysis Using the GeneScan® Software and Windows® Operating Systems

1. Analyze data using the GeneScan® software.

2. Review the raw data for one or more sample runs. Highlight the sample file name, then in the Sample menu, select "raw data". Move the cursor so that the crosshair is on the baseline to the right of the large primer peak (before the first internal lane standard peak [red]). Use the X-value number shown at the bottom left of the window for the start position in the analysis parameters.

3. The recommended analysis parameters are shown in Figure 8.

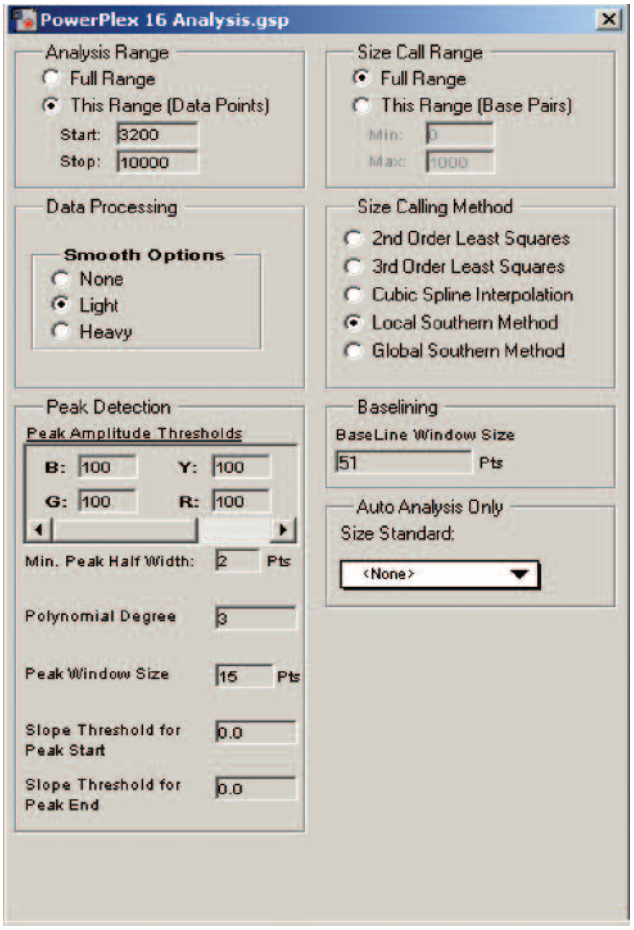

Figure 8. The Analysis Parameters window. The start point of the analysis range, which will vary, is defined in Step 2.

4. The analysis parameters can be saved in the Params folder; in most installations this is located at:
   C:\AppliedBio\Shared\Analysis\Sizecaller\Params
5. Apply the stored analysis parameters file to the samples.
6. Assign a new size standard. Select a sample file, and highlight the arrow next to size standard. Select "define new". Assign the size standard peaks as shown in Figure 13 in Section 9.D. Store the size standard in the Size Standards folder at:
   C:\AppliedBio\Shared\Analysis\Sizecaller\SizeStandards

7. Apply the size standard file to the samples, then analyze the sample files.

Notes:

1. Peak heights outside the linear range of the instrument may generate artifact peaks due to instrument saturation (i.e., overloading the sample). Bleedthrough (pull-ups) from one color to another may be observed. Saturated signal also may appear as two peaks (split peak).
2. If peak heights are not within the linear range of detection of the instrument, the ratio of stutter peaks to real allele peaks increases, and allele designations become difficult to interpret. The balance of peak heights also may appear less uniform.
3. There can be variation between instruments regarding the relative fluorescence levels detected using the same sample. Furthermore, different instruments vary in the relative efficiency of color detection, affecting the dye color-to-dye color balance.

6.F. Sample Analysis Using the Genotyper® Software and PowerTyper™ 16 Macro

To facilitate analysis of data generated with the PowerPlex® 16 System, we have created a file to allow automatic assignment of genotypes using the Genotyper® software. After samples are amplified, detected using the ABI PRISM® 310 or 3100 Genetic Analyzer (using Data Collection Software, Version 1.0.1 or 1.1), and analyzed using the GeneScan® software, sample files can be imported into the Genotyper® program and analyzed using the PowerTyper™ 16 Macro (Release 2.0).

The PowerTyper™ 16 Macro (Release 2.0) can be downloaded from the Promega web site at: **www.promega.com/resources/software-firmware/powertyper-macros/**

The PowerTyper™ 16 Macro (Release 2.0) is used in conjunction with Windows NT® Genotyper® software, version 3.6, or later. The Genotyper® software must be installed on your computer before the PowerTyper™ 16 Macro (Release 2.0) can be used.

Be certain the Color Info (Windows NT® operating systems) column for each lane containing allelic ladder mix contains the word "ladder". The macro uses the word "ladder" to identify the sample file(s) containing allelic ladder. Sample info can be added or modified after importing into the PowerTyper™ Macro. Highlight the sample, then select "show dye/lanes window" in the Views menu.

1. Transfer the PowerTyper™ 16 Macro (Release 2.0) to a designated location on your computer hard drive.
2. Open the Genotyper® software, then the PowerTyper™ 16 Macro (Release 2.0). For questions about the Genotyper® software, refer to the *Genotyper® Analysis Software User's Manual*.

6.F. Sample Analysis Using the Genotyper® Software and PowerTyper™ 16 Macro (continued)

3. In the File menu, select "Import", and import the GeneScan® project or sample files to be analyzed. Import the blue, yellow, green and red dye colors.

   Note: To select the dye colors to be imported, select "Set Preferences" in the Edit menu.

4. Double-click on the Check ILS macro. The macros are listed at the bottom left corner of the active window. A plots window will be displayed to show the internal lane standard (i.e., ILS 600) in the red dye color. Scroll down to view, and confirm that the internal lane standard fragment sizes are correct. If necessary, re-analyze samples using the GeneScan® software and redefine internal lane standard fragments.

   Note: The software uses one ladder sample to determine allele sizes. The macro uses the first ladder sample imported for allele designations.

5. For casework, double-click on the POWER macro. The POWER macro identifies alleles in the ladder sample and calculates offsets for all loci. This process may take several minutes. When completed, a plots window will open to display the allelic ladders (i.e., Penta E, D18S51, D21S11, TH01 and D3S1358).

   Alternatively, for databasing or paternity, double-click on the POWER 20% Filter macro. This macro has a higher level of filtering than the standard POWER macro to reduce the need for manual editing of peak labels. The POWER 20% Filter should not be used if mixtures may exist.

   In general, allelic ladders contain fragments of the same lengths as many known alleles for the locus. Allelic ladder sizes and repeat units are listed in Table 5 (Section 9.A). Analysis using GeneScan® and Genotyper® software allows allele determination by comparing amplified sample fragments with allelic ladders and internal lane standards. When using an internal lane standard, the calculated lengths of allelic ladder components might differ from those listed in the table. This is due to differences in migration resulting from sequence differences between the allelic ladder fragments and internal size standard and is not a matter of concern.

6. Double-click on the Allelic Ladders macro. A plots window will open to display the blue (fluorescein) dye allelic ladders (i.e., Penta E, D18S51, D21S11, TH01 and D3S1358), green (JOE) dye allelic ladders (i.e., Penta E, CSF1PO, D16S539, D7S820, D13S317 and D5S818) and yellow (TMR) dye allelic ladders (i.e., FGA, TPOX, D8S1179, vWA and Amelogenin). Confirm that the correct allele designations were assigned to the allelic ladders (Figure 10 in Section 6.H).

The software uses one ladder sample to determine allele sizes. The macro uses the first ladder sample imported for allele designations. If the POWER macro is run a second time, the software will use the second ladder; if the POWER macro is run a third time, the software will use the third ladder, etc., until all ladders in the project are used. If an allelic ladder fails to be analyzed or if many off-ladder alleles are found in the samples, samples should be re-analyzed using another ladder from the project.

7. Double-click on the Display Fluorescein Data macro to display the blue dye for all sample injections or lanes. Scroll down to observe and edit as needed.

8. Double-click on the Display TMR Data macro to display the yellow dye for all sample injections or lanes. Scroll down to observe and edit as needed.

9. Double-click on the Display JOE Data macro to display the green dye for all sample injections or lanes. Scroll down to observe and edit as needed.

10. Create the appropriate table by selecting the PowerTable, Make Allele Table or Make CODIS Table macro. The three available table formats are shown below. The PowerTable option allows up to four alleles per sample file. Additional information such as low peak signal or high peak signal also is included. The Allele Table and CODIS Table options include only two alleles per locus. If more than two alleles are present at a locus, the smallest alleles identified are included. The Allele Table format displays the categories (loci) in columns, while the CODIS table format displays the categories in rows. These tables can be customized to fit needs. To save data in tables, go to the Table drop-down menu, highlight "Export to File..." and save the file with the desired name and location. The saved file can be viewed and analyzed using Microsoft® Excel.

PowerTable Format

Sample Info	Sample Comment	Category	Peak 1	Peak 2	Peak 3	Peak 4	Over-flow	Low Signal	Satura-tion	Edited Label	Edited Row

Allele Table Format

Sample Info	Category Allele 1	Category Allele 2	Category Allele 1	Category Allele 2	Category Allele 1	Category Allele 2	Category Allele 1	Category Allele 2

CODIS Table Format

Sample Info	Category	Peak 1	Peak 2

11. Save the analyzed data. Go to the File menu, and select "Save as".

The PowerTyper™ Macro is a Genotyper® file and can be overwritten if "Save" is used instead of "Save as".

6.G. 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 Control DNA allele designations for each locus are listed in Table 6 (Section 9.A).

6.H. Results

Representative results of the PowerPlex® 16 System are shown in Figure 9. The PowerPlex® 16 Allelic Ladder Mix is shown in Figure 10.

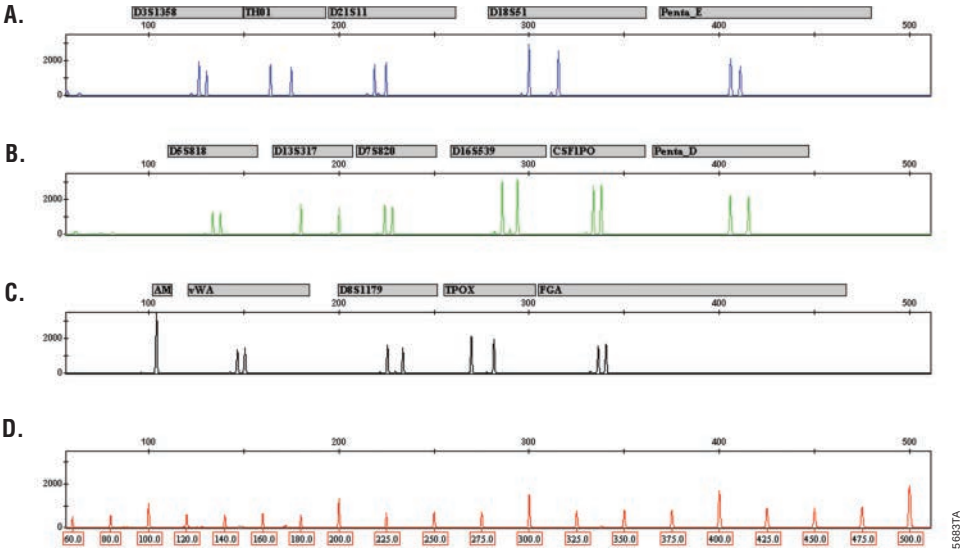

Figure 9. The PowerPlex® 16 System. A single-source template DNA (1.0ng) was amplified using the PowerPlex® 16 System. Amplification products were mixed with Internal Lane Standard 600 and analyzed with an Applied Biosystems 3130 Genetic Analyzer using a 3kV, 5-second injection. Results were analyzed using GeneMapper® *ID* software, version 3.2. **Panel A.** An electropherogram showing the peaks of the fluorescein-labeled loci: D3S1358, TH01, D21S11, D18S51 and Penta E. **Panel B.** An electropherogram showing the peaks of the JOE-labeled loci: D5S818, D13S317, D7S820, D16S539, CSF1PO and Penta D. **Panel C.** An electropherogram showing the peaks of the TMR-labeled loci: Amelogenin, vWA, D8S1179, TPOX and FGA. **Panel D.** An electropherogram showing the 60bp to 500bp fragments of the Internal Lane Standard 600.

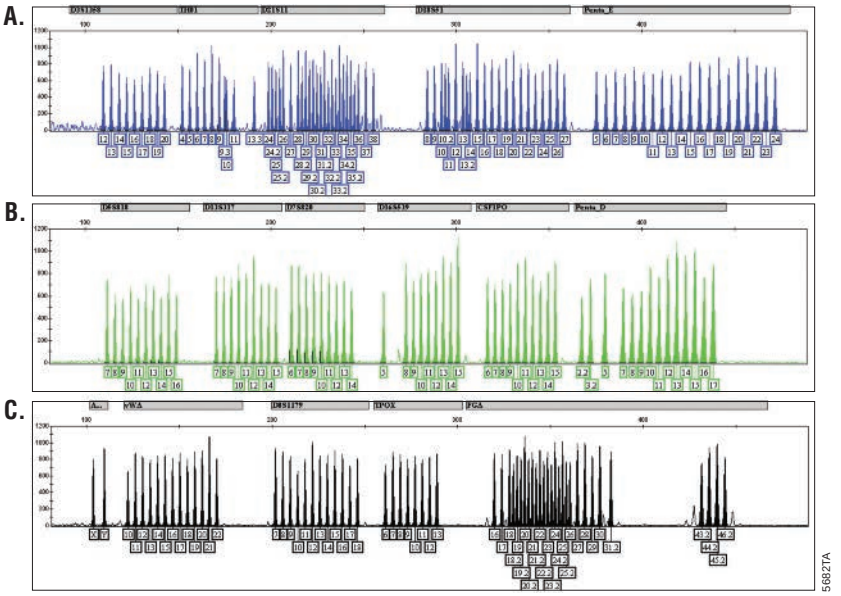

Figure 10. The PowerPlex® 16 Allelic Ladder Mix. The PowerPlex® 16 Allelic Ladder Mix was analyzed with an Applied Biosystems 3130 Genetic Analyzer using a 3kV, 5-second injection. The sample file was analyzed with the GeneMapper® *ID* software, version 3.2, and PowerPlex® 16 panels and bins text files. **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-labeled allelic ladder components and their allele designations.

Artifacts and Stutter

Stutter products are a common amplification artifact associated with STR analysis. Stutter products often are observed one repeat unit below the true allele peak and, occasionally, two repeat units smaller or one repeat unit larger than the true allele peak. Frequently, alleles with a greater number of repeat units will exhibit a higher percent stutter. The pattern and intensity of stutter may differ slightly between primer sets for the same loci. The level of stutter was determined and published as part of the PowerPlex® 16 System validation (9).

In addition to stutter peaks, other artifact peaks can be observed at some of the PowerPlex® 16 System loci. Low-level products can be seen in the n–2 and n+2 positions (two bases below and above the true allele peak, respectively) with some loci such as D21S11. Samples may show low-level artifacts in the noncalling regions between the D7S820 and D13S317 allele ranges and between the D3S1358 and TH01 allele ranges. Occasionally an off-ladder artifact can be seen in the 270–271bp position in the JOE dye channel. One or more extra peaks that are not directly related to amplification may be observed at positions 8–26 bases smaller than TPOX alleles and 6–21 bases smaller than vWA alleles. These extra peaks occur when the amplified peaks are particularly intense (high signal intensity or template amount); formamide, polymer or capillary was of poor quality; or denaturation was ineffective. See Section 7 for more information about how to minimize these artifacts.

A low-level artifact in the D5S818 region of the JOE channel may be observed at 114–120bp. In addition, low-level artifacts in the TMR channel may be observed at 142–144 and 400–405bp

These artifacts are not template-derived and may appear in the negative control and in low-product-yield analyses. The peak heights of these artifacts may increase with longer injection time or higher injection voltage.

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.
	Insufficient enzyme activity. Use the recommended amount of AmpliTaq Gold® DNA polymerase. Check the expiration date on the tube label.
	Incorrect amplification program. Confirm the amplification program.
	An air bubble formed at the bottom of the reaction well. Use a pipette to remove the air bubble, or centrifuge the reactions briefly before thermal cycling.
	High salt concentration or altered pH. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the DNA volume should not exceed 20% of the total reaction volume. Carryover of K^+, Na^+, Mg^{2+} or EDTA from the DNA sample can negatively affect PCR. A change in pH also may affect PCR. Store DNA in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA), TE^{-4} buffer with 20µg/ml glycogen or nuclease-free water.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl. Decreasing the reaction volume may result in suboptimal performance.
	Thermal cycler, plate or tube problems. Review the thermal cycling protocols 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 10X PowerPlex® 16 Primer Pair for 15 seconds before use.

Symptoms	Causes and Comments
Faint or absent allele peaks (continued)	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 electrophoresis. Do not cool samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing.
	Poor capillary electrophoresis injection (ILS 600 peaks also affected). Re-inject the sample. Check the instrument syringe pump system for leakage.
	Poor capillary electrophoresis injection (ILS 600 peaks also affected). Check the laser power.
	Poor-quality formamide was used. Use only Hi-Di™ formamide when analyzing samples.
Extra peaks visible in one or all color channels	Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips, and change gloves regularly.
	Samples were not completely denatured. Heat denature samples for the recommended time, and cool on crushed ice or in an ice-water bath immediately prior to electrophoresis. Do not cool samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing.
	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as faint peaks one repeat unit smaller than the allele. Stutter product peak heights can be high if samples are overloaded. See Section 6.H for additional information about stutter and artifacts.
	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´ A residue. Be sure to perform the 30-minute extension step at 60°C after thermal cycling (Section 4).
	Excessive amount of DNA. Amplification of >2ng template can result in a higher number of artifact peaks. Use less template DNA, or reduce the number of cycles in the amplification program (e.g. 10/20 or 10/18 cycling).
	High background. Load less amplification product, or decrease injection time. See Section 5.
	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.
	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 blue and green dye colors. Use autoclaved deionized water; change vials and wash buffer reservoir.

7.A. Amplification and Fragment Detection (continued)

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels (continued)	Pull-up or bleedthrough. Pull-up can occur when peak heights are too high or if a poor or incorrect matrix has been applied to the samples. • For the ABI PRISM® 310 Genetic Analyzer, generate a new matrix, and apply it to the samples. For the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130 and 3130*xl* Genetic Analyzers, perform a new spectral calibration and re-run the samples. • Instrument sensitivities can vary. Optimize the injection conditions. See Section 5.
	Repeat sample preparation using fresh formamide. Long-term storage of amplified sample in formamide can result in artifacts.
	The CE polymer was beyond its expiration date, or polymer was stored at room temperature for more than one week.
	Maintain instrumentation on a daily or weekly basis, as recommended by the manufacturer.
Allelic ladder not running the same as samples	Allelic ladder and primer pair mix were not compatible. Ensure that the allelic ladder is from the same kit as the primer pair mix.
	Buffer incompatibility. Samples were diluted in the wrong buffer. Use Gold ST★R 1X Buffer to dilute samples.
	Poor-quality formamide. Use only Hi-Di™ formamide when analyzing samples.
	Be sure the allelic ladder and samples are from the same instrument run.
	Migration of samples changed slightly over the course of a CE run with many samples. This may be due to changes in temperature or the CE column over time. Use a different injection of allelic ladder to determine sizes.
	Poor injection of allelic ladder. Include more than one ladder per instrument run.
Peak height imbalance	Excessive amount of DNA. Amplification of >1ng of template can result in an imbalance, with smaller loci showing more product than larger loci. Use less template, or reduce the number of cycles in the amplification program by (e.g. 10/20 or 10/18 cycling) to improve locus-to-locus balance. **Note:** Dilution of overamplified samples can result in dropout of larger loci.
	Degraded DNA sample. DNA template was degraded, and larger loci show diminished yield. Repurify template DNA.
	Insufficient template DNA. Use the recommended amount of template DNA. Stochastic effects can occur when amplifying low amounts of template.
	Miscellaneous balance problems. Thaw the 10X Primer Pair Mix and Gold ST★R 10X Buffer completely, and vortex for 15 seconds before using. Do not centrifuge the 10X Primer Pair Mix after mixing. Calibrate thermal cyclers and pipettes routinely. Using a 59°C annealing temperature instead of 60°C has been shown to improve balance in some instances.

Symptoms	Causes and Comments
Peak height imbalance (continued)	PCR amplification mix prepared in Section 4 was not mixed well. Vortex the PCR amplification mix for 5–10 seconds before dispensing into reaction tubes or plate.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl. Decreasing the reaction volume can result in suboptimal performance.
	Impure template DNA. Inhibitors that may be present in forensic samples can lead to allele dropout or imbalance.

7.B. Direct Amplification of DNA from NonFTA 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	DNA was not accessible on nonlytic material. Pretreat nonFTA materials with PunchSolution™ Reagent to ensure that DNA is liberated from cellular proteins.
	Poor sample deposition. Shedding and collection of donor cells was variable. Increase cycle number.
	Poor sample transfer to storage card or variable sampling from the storage card. Take punches from a different portion of the card. Increasing cycle number also can improve low peak heights.
	Too much sample in the reaction. Use one 1.2mm nonFTA storage card punch. Follow the manufacturer's recommendations when depositing sample onto the storage card.
	Blood card punches were used. We do not recommend analysis of blood card punches.
	Make sure that the PCR amplification mix contained AmpSolution™ Reagent. Omission of AmpSolution™ Reagent from amplification reactions will result in amplification failure.
	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.
	Inactive PunchSolution™ Reagent. Thaw the PunchSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.
Faint or absent peaks for the positive control reaction	If the positive control reaction failed to amplify, check to make sure that the correct amount of 2800M Control DNA was added to the reaction. Optimize the amount of 2800M Control DNA for your thermal cycling conditions and laboratory preferences.
	Improper storage of the 2800M Control DNA.

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

Symptoms	Causes and Comments
Extra peaks visible in one or or all color channels	Punch was contaminated. Take punches from blank paper samples, and include a reaction with one blank punch as a negative control.
	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. We recommend one 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.
	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 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.
	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.H for additional information about stutter and artifacts.
Peak height imbalance	Excessive amount of DNA. Amplification of >20ng of template can result in an imbalance with smaller loci showing more product than larger loci. • Use one 1.2mm punch from a nonFTA card containing a buccal sample. Follow the manufacturer's recommendations when depositing sample onto the storage card. • Decrease cycle number.
	Amplification was inhibited when using more than one storage card punch. Use only one 1.2mm storage card punch.
	Active PunchSolution™ Reagent carried over into the amplification reaction. Larger loci are most susceptible to carryover and will drop out before smaller loci. • Ensure that the heat block reached 70°C and samples were incubated for 30 minutes. 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.

Symptoms	Causes and Comments
Peak height imbalance (continued)	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.
	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.
Extreme variability in sample-to-sample peak heights	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.

7.C. Direct 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	Poor sample deposition. Shedding and collection of donor cells was variable. Increase cycle number.
	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.
	Make sure that the PCR amplification mix contained AmpSolution™ Reagent. Omission of AmpSolution™ Reagent from amplification reactions will result in amplification failure.
	Active SwabSolution™ Reagent carried over into the amplification reaction. Ensure that the heat block is heating to 70°C (90°C if using a 2.2ml, Square-Well Deep Well Plate) and samples were incubated for the full 30 minutes. Incubation for shorter time periods may result in incomplete reagent inactivation. Do not use an incubator to incubate tubes or plates: Heat transfer is inefficient and will result in poor performance. 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.

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

Symptoms	Causes and Comments
Faint or absent peaks for the positive control reaction	If the positive control reaction failed to amplify, check to make sure that the correct amount of 2800M Control DNA was added to the reaction. Due to the reduced cycle 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.
Extra peaks visible in one or all color channels	Swab extract was contaminated. Include a blank swab as a negative control when processing samples.
	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.
	Amplification of excess template for a given cycle number resulted in overloading of the capillary upon electrokinetic injection. The presence of excess DNA in the capillary makes it difficult to maintain 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 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	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.
	Active SwabSolution™ Reagent carried over into the amplification reaction. Larger loci are most susceptible to reagent carryover and will drop out before smaller loci. Ensure that the heat block is heating to 70°C (90°C if using 2.2ml, Square-Well Deep Well Plates) and samples were incubated for the full 30 minutes. Incubation for shorter time periods may result in incomplete reagent inactivation. Do not use an incubator to incubate tubes or plates. Heat transfer is inefficient and will result in poor performance. Use only a heat block to maintain efficient heat transfer.
	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.

Symptoms	Causes and Comments
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. The quantitation values can be used to normalize input template amounts to minimize variation in signal intensity.
	DNA was not accessible on nonlytic material. Pretreat swabs with SwabSolution™ Reagent to ensure that DNA is liberated from cellular proteins.

7.D. GeneMapper® *ID* Software

Symptoms	Causes and Comments
Alleles not called	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 (Figure 11).
	To analyze samples with GeneMapper® *ID* software, at least one allelic ladder must be defined.
	An insufficient number of ILS 600 fragments was defined. Be sure to define at least one ILS 600 fragment smaller than the smallest sample peak or allelic ladder peak and at least one ILS 600 fragment larger than the largest sample peak or allelic ladder peak.
	Run was too short, and larger peaks in ILS were not captured. Not all ILS 600 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.

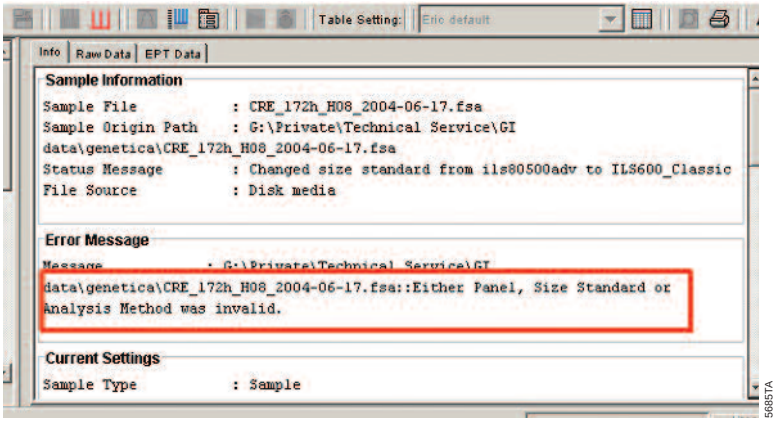

Figure 11. The error message that appears in the GeneMapper® *ID* software when the analysis parameters and size standard have different analysis types.

Symptoms	Causes and Comments
Off-ladder alleles	An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.
	The GeneMapper® *ID* software requires that the allelic ladder be imported from the same folder as the sample. Be sure that the allelic ladder is in the same folder as the sample. Create a new project and re-analyze, as described in Section 6.C or 6.D.
	Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file that corresponds to the STR system used for amplification.
	The allelic ladder was not identified as an allelic ladder in the Sample Type column.
	The wrong analysis type was chosen for the analysis method. Be sure to use the HID analysis type.
	The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.
Size standard not called correctly (Figure 12)	Starting data point was incorrect for the partial range chosen in Section 6.C. Adjust the starting data point in the analysis method. Alternatively, use a full range for the analysis.
	Extra peaks in advanced mode size standard. Open the Size Match Editor. Highlight the extra peak, select "Edit" and select "delete size label". Select "auto adjust sizes".
	Run was too short, and larger peaks in ILS were not captured. Not all ILS 600 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.

Figure 12. An example showing improper assignment of size standard fragments in the GeneMapper® *ID* software.

Symptoms	Causes and Comments
Peaks in size standard missing	If peaks are below threshold, decrease the peak amplitude threshold in the analysis method for the red channel to include peaks.
	If peaks are low-quality, redefine the size standard for the sample to skip these peaks.
Error message: "Either panel, size standard, or analysis method is invalid"	The size standard and analysis method were not in the same mode ("Classic" vs. "Basic or Advanced"). Be sure both files are set to the same mode, either Classic or Basic or Advanced mode.
No alleles called, but no error message appears	Panels text file was not selected for sample. In the Panel column, select the appropriate panels text file for the STR system that was used.
	No size standard was selected. In the Size Standards column, be sure to select the appropriate size standard.
	Size standard was not correctly defined, or size peaks were missing. Redefine size standard to include only peaks present in your sample. Terminating analysis early or using short run times will cause larger ladder peaks to be missing. This will cause your sizing quality to be flagged as "red", and no allele sizes will be called.
Error message: "Both the Bin Set used in the Analysis Method and the Panel must belong to the same Chemistry Kit"	The bins text file assigned to the analysis method was deleted. In the GeneMapper® Manager, select the Analysis Methods tab, and open the analysis method of interest. Select the Alleles tab, and select the appropriate bins text file.
	The wrong bins text file was chosen in the analysis method Allele tab. Be sure to choose the appropriate bins text file, as shown in Figure 5.
Significantly raised baseline	• Poor spectral calibration for the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130 and 3130*xl* 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.
	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.
Red bar appears during analysis of samples, and the following error message appears when data are displayed: "Some selected sample(s) do not contain analysis data. Those sample(s) will not be shown".	If none of the samples had matrices applied when run on the ABI PRISM® 310 Genetic Analyzer, no data will be displayed. Apply a matrix file during analysis in the GeneMapper® *ID* software and re-analyze.

7.D. GeneMapper® *ID* Software (continued)

Symptoms	Causes and Comments
Error message after attempting to import panels and bins text files: "Unable to save panel data: java.SQLEException: ORA-00001: unique constraint (IFA.CKP_NNN) violated".	There was a conflict between different sets of panels and bins text files. Check to be sure that the bins are installed properly. If not, delete all panels and bins text files, and re-import files in a different order.
Allelic ladder peaks are labeled off-ladder	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.

7.E. PowerTyper™ 16 Macro

Symptoms	Causes and Comments
File does not open on your computer	Genotyper® software was not installed. Be certain that the Genotyper® software, version 3.6 or higher (Windows NT®), is installed.
	Incorrect version of Genotyper® software. The PowerTyper™ 16 Macro will not work with Genotyper® software versions prior to version 2.5.
Error message: "Could not complete the "Run Macro" command because no dye/lanes are selected"	Allelic ladder sample files were not identified. Be certain the Sample Info or Color Info column for each lane containing PowerPlex® 16 Allelic Ladder Mix contains the word "ladder". The macro uses the word "ladder" to identify sample files containing allelic ladder.
	All four dye colors were not imported. For Genotyper® software, versions 2.5 and 3.5 or higher, set preferences (in the Edit menu) to import the blue, green, yellow and red colors.
Error message: "Could not complete the "Run Macro" command because the labeled peak could not be found"	Peak heights for one or more alleles in the allelic ladder sample file were below 150RFU. The allelic ladder categories are defined as having a minimum peak height of 150RFU. If peak heights of ladder alleles are below 150RFU, the software will not be able to locate the allele peak. Re-run the allelic ladder using more sample or longer injection time to ensure that peak heights are above 150RFU.
	CE spikes in the allelic ladder sample were identified as alleles by the macro. Use a different injection of allelic ladder.
	TH01 9.3 and 10 alleles were not separated when using heavy smoothing in the GeneScan® analysis parameters. Use light smoothing in the GeneScan® analysis parameters.
	Allelic ladder data were not compatible with the PowerTyper™ file used. Confirm that the PowerTyper™ Macro file matches the allelic ladder being used.

Symptoms	Causes and Comments
Error message: "Could not complete the "Run Macro" command because the labeled peak could not be found"(continued)	The base-pair size of alleles in the allelic ladder are outside of the defined category range. Be sure internal lane standard fragments are correctly sized. Redefine internal lane standard fragments, and re-analyze the sample using GeneScan® software. Compare the size of the smallest allele in the allelic ladder with the base-pair size and range listed in the categories for the same alleles. If necessary, increase the category start range (in the category window) to greater than ±6bp, and save the macro under a new name.
	Allelic ladder peaks were too high, causing stutter peaks to be called as allele peaks. Use a shorter injection time, decrease the amount of allelic ladder used or re-analyze the allelic ladder sample using increased peak amplitude thresholds in the GeneScan® analysis parameters.
	Allelic ladder data were not compatible with the PowerTyper™ Macro file used. Confirm that the PowerTyper™ Macro file matches the allelic ladder being used.
The plots window or allele table does not display all data	The macros were not run in the proper order. Use the POWER or POWER 20% Filter macro option.
	All four dye colors were not imported. For Genotyper® software, versions 2.5 and 3.5 or higher, set preferences (in the Edit menu) to import the blue, green, yellow and red colors.
The Check ILS macro displays an empty plot window	All four dye colors were not imported. For Genotyper® software, versions 2.5 and 3.5 or higher, set preferences (in the Edit menu) to import the blue, green, yellow and red colors.
Off-ladder peaks	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 in the PowerTyper™ 16 Macro (Release 2.0). Do not use the first injection on a new column for the ladder sample.
	The base-pair size of alleles was incorrect because incorrect fragment sizes were assigned to the internal lane standard. Confirm that internal lane standard fragment sizes are assigned correctly. Re-analyze the sample using GeneScan® software, and redefine the internal lane standard fragments.

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9. Appendix

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

The loci included in the PowerPlex® 16 System (Tables 4 and 5) were selected because they satisfy the needs of several major standardization bodies throughout the world. For example, the United States Federal Bureau of Investigation (FBI) has selected 13 STR core loci for typing prior to searching or including (submitting) samples in CODIS (Combined DNA Index System), the U.S. national database of convicted offender profiles. The PowerPlex® 16 System amplifies the 13 CODIS core loci in a single reaction.

The PowerPlex® 16 System also contains two low-stutter, highly polymorphic pentanucleotide repeat loci: Penta E and Penta D. These additional loci add significantly to the discrimination power of the system, making the PowerPlex® 16 System a single-amplification system with a power of exclusion sufficient to resolve paternity disputes definitively. In addition, the extremely low level of stutter seen with Penta E and Penta D makes them ideal loci to evaluate DNA mixtures often encountered in forensic casework. Finally, the Amelogenin locus is included in the PowerPlex® 16 System to allow gender identification of each sample. Table 6 lists the PowerPlex® 16 System alleles revealed in commonly available standard DNA templates.

We have carefully selected STR loci and primers to avoid or minimize artifacts, including those associated with *Taq* DNA polymerase, such as repeat slippage and terminal nucleotide addition. Repeat slippage (15,16), sometimes called "n–4 peaks", "stutter" or "shadow bands", is due to the loss of a repeat unit during DNA amplification, somatic variation within the DNA, or both. The amount of this artifact observed depends primarily on the locus and the DNA sequence being amplified.

Terminal nucleotide addition (17,18) 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 for 30 minutes (19) to the amplification protocols to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of template DNA are used.

The presence of microvariant alleles (alleles differing from one another by lengths other than the repeat length) complicates interpretation and assignment of alleles. There appears to be a correlation between a high degree of polymorphism, a tendency for microvariants and increased mutation rate (20,21). Thus, FGA and D21S11 display numerous, relatively common microvariants. For reasons yet unknown, the highly polymorphic Penta E locus does not display frequent microvariants (Table 5).

Table 4. The PowerPlex® 16 System Locus-Specific Information.

STR Locus	Label	Chromosomal Location	GenBank® Locus and Locus Definition	Repeat Sequence[1] 5′→ 3′
Penta E	FL	15q	NA	AAAGA
D18S51	FL	18q21.3	HUMUT574	AGAA (21)
D21S11	FL	21q11–21q21	HUMD21LOC	TCTA Complex (21)
TH01	FL	11p15.5	HUMTH01, human tyrosine hydroxylase gene	AATG (21)
D3S1358	FL	3p	NA	TCTA Complex
FGA	TMR	4q28	HUMFIBRA, human fibrinogen alpha chain gene	TTTC Complex (21)
TPOX	TMR	2p24–2pter	HUMTPOX, human thyroid peroxidase gene	AATG
D8S1179	TMR	8q24.13	NA	TCTA Complex (21)
vWA	TMR	12p13.31	HUMVWFA31, human von Willebrand factor gene	TCTA Complex (21)
Amelogenin[2]	TMR	Xp22.1–22.3 and Y	HUMAMEL, human Y chromosomal gene for Amelogenin-like protein	NA
Penta D	JOE	21q	NA	AAAGA
CSF1PO	JOE	5q33.3–34	HUMCSF1PO, human c-fms proto-oncogene for CSF-1 receptor gene	AGAT
D16S539	JOE	16q24.1	NA	GATA
D7S820	JOE	7q11.21–22	NA	GATA
D13S317	JOE	13q22–q31	NA	TATC
D5S818	JOE	5q23.3–32	NA	AGAT

[1]The August 1997 report (23,24) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, "1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5′ nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used".

[2]Amelogenin is not an STR.

TMR = carboxy-tetramethylrhodamine

FL = fluorescein

JOE = 6-carboxy-4′,5′-dichloro-2′,7′-dimethoxyfluorescein

NA = not applicable

Table 5. The PowerPlex® 16 System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components[1,2] (bases)	Repeat Numbers of Allelic Ladder Components	Repeat Numbers of Alleles Not Present in Allelic Ladder[3,4]
Penta E	FL	379–474	5–24	20.3
D18S51	FL	290–366	8–10, 10.2, 11–13, 13.2, 14–27	
D21S11	FL	203–259	24, 24.2, 25, 25.2, 26–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38	
TH01	FL	156–195	4–9, 9.3, 10–11, 13.3	
D3S1358	FL	115–147	12–20	
FGA	TMR	322–444	16–18, 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, 43.2, 44.2, 45.2, 46.2	
TPOX	TMR	262–290	6–13	
D8S1179	TMR	203–247	7–18	
vWA	TMR	123–171	10–22	
Amelogenin[5]	TMR	106, 112	X, Y	
Penta D	JOE	376–449	2.2, 3.2, 5, 7–17	
CSF1PO	JOE	321–357	6–15	
D16S539	JOE	264–304	5, 8–15	
D7S820	JOE	215–247	6–14	
D13S317	JOE	176–208	7–15	
D5S818	JOE	119–155	7–16	

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

[2]When using an internal lane standard, such as the Internal Lane Standard 600, the calculated sizes of allelic ladder components may differ from those listed. This occurs because different sequences in allelic ladder and ILS components may cause differences in migration. The dye label also affects migration of alleles.

[3]The alleles listed are those with a frequency of >1/1000.

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

[5]Amelogenin is not an STR.

Table 6. The PowerPlex® 16 System Allele Determinations in Commonly Available Standard DNA Templates.

	Standard DNA Templates[1]	
STR Locus	**K562[1]**	**2800M**
Penta E	5, 14	7, 14
D18S51	15, 16	16, 18
D21S11	29, 30, 31	29, 31.2
TH01	9.3, 9.3	6, 9.3
D3S1358	16, 16	17, 18
FGA	21, 24	20, 23
TPOX	8, 9	11, 11
D8S1179	12, 12	14, 15
vWA	16, 16	16, 19
Amelogenin	X, X	X, Y
Penta D	9, 13	12, 13
CSF1PO	9, 10	12, 12
D16S539	11, 12	9, 13
D7S820	9, 11	8, 11
D13S317	8, 8	9, 11
D5S818	11, 12	12, 12

[1]Strain K562 is available from the American Type Culture Collection: **www.atcc.org** (Manassas, VA).

[2]Strain K562 displays three alleles at the D21S11 locus.

9.B. Power of Discrimination

The fifteen STR loci amplified with the PowerPlex® 16 System provide powerful discrimination. Population statistics for these loci and their various multiplex combinations are displayed in Table 7. These data were generated as part of a collaboration (25) with The Bode Technology Group (Springfield, VA), North Carolina Bureau of Investigation (Raleigh, NC), Palm Beach County Sheriff's Office (West Palm Beach, FL), Virginia Division of Forensic Science (Richmond, VA) and Charlotte/Mecklenburg Police Department Laboratory (NC). Data generation included analysis of over 200 individuals from African-American, Caucasian-American and Hispanic-American populations. Data for Asian-Americans include analysis of more than 150 individuals. For additional population data for STR loci, see references 26–31 and the Short Tandem Repeat DNA Internet DataBase at: **www.cstl.nist.gov/div831/strbase/**

Table 7 shows the matching probability (32) for the PowerPlex® 16 System in various populations. The matching probability ranges from 1 in 1.83×10^{17} for Caucasian-Americans to 1 in 1.41×10^{18} for African-Americans.

A measure of discrimination often used in paternity analyses is the paternity index (PI), a means for presenting the genetic odds in favor of paternity given the genotypes for the mother, child and alleged father (33). The typical paternity indices for the PowerPlex® 16 System are shown in Table 7. The PowerPlex® 16 System provides typical paternity indices exceeding 500,000 in each population group. An alternative calculation used in paternity analyses is the power of exclusion (33). This value, calculated for the PowerPlex® 16 System, exceeds 0.999998 in all populations tested.

Table 7. Matching Probabilities, Paternity Indices and Power of Exclusion of the PowerPlex® 16 System in Various Populations.

	African-American	Caucasian-American	Hispanic-American	Asian-American
Matching Probability	1 in 1.41×10^{18}	1 in 1.83×10^{17}	1 in 2.93×10^{17}	1 in 3.74×10^{17}
Paternity Index	2,510,000	1,520,000	522,000	4,110,000
Power of Exclusion	0.9999996	0.9999994	0.9999983	0.9999998

9.C. 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.

For analysis of database, reference and other single-source samples, we recommend preprocessing of swabs and nonFTA punches with the SwabSolution™ Kit or PunchSolution™ Kit, respectively. The SwabSolution™ Kit (Cat.# DC8271) contains reagents for rapid DNA preparation from buccal swabs prior to amplification. The procedure lyses cells contained on the swab head and releases into solution sufficient DNA for STR amplification. A small volume of the final swab extract is added to the PowerPlex® reaction. The PunchSolution™ Kit is used to process punches from nonFTA storage cards containing blood or buccal samples prior to direct amplification. When performing direct amplification with the PowerPlex® 16 System, make sure that the PCR amplification mix contains AmpSolution™ Reagent. Omission of AmpSolution™ Reagent from amplification reactions will result in amplification failure.

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.

For casework or samples that require DNA purification, we recommend the DNA IQ™ System (Cat.# DC6700), which is a DNA isolation system designed specifically for forensic and paternity samples (34). This system uses paramagnetic particles to prepare clean samples for STR analysis easily and efficiently and can be used to extract DNA from stains or liquid samples, such as blood or solutions. The DNA IQ™ Resin eliminates PCR inhibitors and contaminants frequently encountered in casework samples. With DNA-rich samples, the DNA IQ™ System delivers a consistent amount of total DNA. The system has been used to isolate DNA from routine sample types including buccal swabs, stains on FTA® paper and liquid blood. Additionally, DNA has been isolated from casework samples such as tissue, differentially separated sexual assault samples and stains on support materials. The DNA IQ™ System has been tested with the PowerPlex® Systems to ensure a streamlined process. See Section 9.F for ordering information.

For applications requiring human-specific DNA quantification, the Plexor® HY System (Cat.# DC1000) was developed (35). See Section 9.F for ordering information.

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/**

9.D. The Internal Lane Standard 600

The Internal Lane Standard (ILS) 600 contains 22 DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases in length (Figure 13). Each fragment is labeled with carboxy-X-rhodamine (CXR) and can be detected separately (as a fourth color) in the presence of PowerPlex® 16-amplified material. The ILS 600 is designed for use in each CE injection to increase precision in analyses when using the PowerPlex® 16 System. Protocols for preparation and use of this internal lane standard are provided in Section 5.

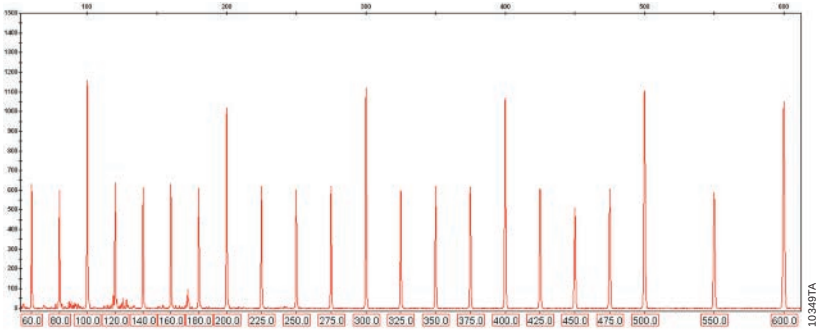

Figure 13. Internal Lane Standard 600. An electropherogram showing the Internal Lane Standard 600 fragments.

9.E. Composition of Buffers and Solutions

Blue Dextran Loading Solution

88.25%	formamide
15mg/ml	blue dextran
4.1mM	EDTA (pH 8.0)

Gold ST★R 10X Buffer

500mM	KCl
100mM	Tris-HCl (pH 8.3 at 25°C)
15mM	$MgCl_2$
1%	Triton® X-100
2mM	each dNTP
1.6mg/ml	BSA

TAE 50X buffer (pH 7.2)

242g	Tris base
57.1ml	glacial acetic acid
100ml	0.5M EDTA stock

Add Tris base and EDTA stock to 500ml of deionized water. Add glacial acetic acid. Bring the volume to 1 liter with deionized water.

TBE 10X buffer

107.8g	Tris base
7.44g	EDTA ($Na_2EDTA \cdot 2H_2O$)
~55.0g	boric acid

Dissolve Tris base and EDTA in 800ml of deionized water. Slowly add the boric acid, and monitor the pH until the desired pH of 8.3 is obtained. Bring the final volume to 1 liter with deionized water.

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

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

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

TE^{-4} buffer with 20µg/ml glycogen

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

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

9.F. Related Products

STR Systems

Product	Size	Cat.#
PowerPlex® 16 Monoplex System, Penta E (Fluorescein)	100 reactions	DC6591
PowerPlex® 16 Monoplex System, Penta D (JOE)	100 reactions	DC6651
PowerPlex® Fusion System	200 reactions	DC2402
	800 reactions	DC2408
PowerPlex® 21 System	200 reactions	DC8902
PowerPlex® 16 HS System	100 reactions	DC2101
	400 reactions	DC2100
PowerPlex® ESX 17 Fast System	100 reactions	DC1711
	400 reactions	DC1710
PowerPlex® ESI 17 Fast System	100 reactions	DC1721
	400 reactions	DC1720
PowerPlex® ESX 16 System	100 reactions	DC6711
	400 reactions	DC6710
PowerPlex® ESI 16 System	100 reactions	DC6771
	400 reactions	DC6770
PowerPlex® Y23 System	50 reactions	DC2305
	200 reactions	DC2320

Not for Medical Diagnostic Use.

Accessory Components

Product	Size	Cat.#
PowerPlex® Matrix Standards, 310*	50µl (each dye)	DG4640
PowerPlex® 4C Matrix Standard*	5 preps	DG4800
PunchSolution™ Kit*	100 preps	DC9271
SwabSolution™ Kit*	100 preps	DC8271
Internal Lane Standard 600	150µl	DG1071
2800M Control DNA (10ng/µl)	25µl	DD7101
2800M Control DNA (0.25ng/µl)	500µl	DD7251
Water, Amplification Grade	5 × 1,250µl	DW0991
Gold ST★R 10X Buffer	1.2ml	DM2411

*Not for Medical Diagnostic Use.

Sample Preparation and DNA Quantitation Systems

Product	Size	Cat.#
DNA IQ™ System	100 reactions	DC6701
	400 reactions	DC6700
Differex™ System*	50 samples	DC6801
	200 samples	DC6800
Maxwell® 16 Forensic Instrument*	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
PowerQuant™ System	800 reactions	PQ5008
	200 reactions	PQ5002
Slicprep™ 96 Device	10 pack	V1391

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

10. Summary of Changes

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

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

[a]U.S. Pat. No. 6,238,863, Chinese Pat. No. ZL99802696.4, European Pat. No. 1058727, Japanese Pat. No. 4494630 and other patents pending.

[b]Australian Pat. No. 724531, Canadian Pat. No. 2,251,793, Korean Pat. No. 290332, Singapore Pat. No. 57050, Japanese Pat. Nos. 3602142 and 4034293, Chinese Pat. Nos. ZL99813729.4 and ZL97194967.0, European Pat. No. 0960207 and other patents pending.

[c]The purchase of this product does not convey a license to use AmpliTaq Gold® DNA polymerase. You should purchase AmpliTaq Gold® DNA polymerase licensed for the forensic and human identity field directly from your authorized enzyme supplier.

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