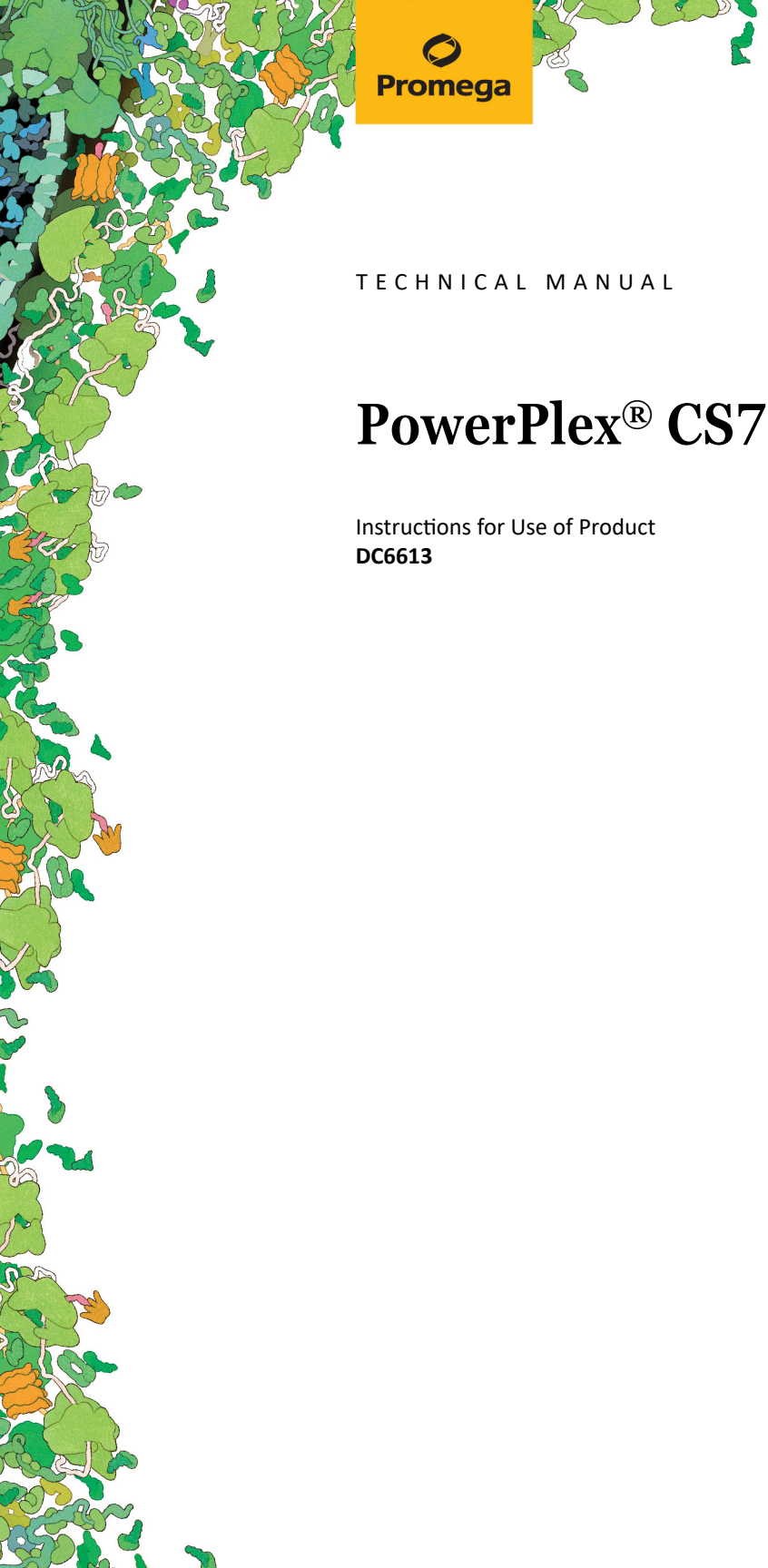

TECHNICAL MANUAL

PowerPlex® CS7 System

Instructions for Use of Product
DC6613

Revised 7/22
TMD042

Promega

PowerPlex® CS7 System

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

Promega

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® CS7 System[a,b] is used for human identification applications and research use. The system allows co-amplification and three-color detection of seven STR loci, including LPL, F13B, FESFPS, F13A01, Penta D, Penta C and Penta E. One primer for each of the LPL, F13B, FESFPS, F13A01 and Penta D loci is labeled with fluorescein (FL); one primer for the Penta E locus is labeled with carboxytetramethylrhodamine (TMR); and one primer for the Penta C locus is labeled with 6-carboxy-4´,5´-dichloro-2´,7´-dimethoxy-fluorescein (JOE). All seven loci are amplified simultaneously in a single tube and analyzed in a single injection. The PowerPlex® CS7 System contains two loci that overlap with loci included in the PowerPlex® 16 HS System: Penta D and Penta E. This feature allows the PowerPlex® CS7 System to be used as a confirmatory kit in paternity applications using the five unshared STR loci to supplement the genotype and increase the available information.

The PowerPlex® CS7 System provides all materials necessary to amplify STR regions of purified human genomic DNA. This manual contains separate protocols for use of the PowerPlex® CS7 System with GeneAmp® PCR System 9700 and Applied BioSystems ProFlex® PCR System thermal cyclers in addition to protocols to separate amplified products and detect separated material. 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**

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
PowerPlex® CS7 System	**100 reactions**	**DC6613**

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

Pre-amplification Components Box
- 500µl PowerPlex® HS 5X Master Mix
- 250µl PowerPlex® CS7 10X Primer Pair Mix
- 25µl 2800M Control DNA, 10ng/µl
- 2 × 1.25ml Water, Amplification Grade

Post-amplification Components Box
- 50µl PowerPlex® CS7Allelic Ladder Mix
- 150µl Internal Lane Standard 600

The PowerPlex® CS7 Allelic Ladder Mix, is provided in a separate, sealed bag for shipping. This component should be moved to the post-amplification box after opening.

PowerPlex® HS 5X Master Mix and PowerPlex® CS7 10X Primer Pair Mix are manufactured as a matched set for optimal performance. Do not combine components from kits with different lot numbers (printed on the boxes and Certificates of Analysis). If lots are mixed, locus-to-locus imbalance and variation in signal intensity may occur.

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°C to +10°C. Make sure that the 2800M Control DNA is stored at +2°C to +10°C for at least 24 hours before use. The PowerPlex® CS7 10X Primer Pair Mix, PowerPlex® CS7 Allelic Ladder Mix and Internal Lane Standard 600 (ILS 600) are light-sensitive and must be stored in the dark. We strongly recommend that pre-amplification and post-amplification reagents be stored and used separately with different pipettes, tube racks, etc.

Available Separately

The proper panels and bins text files for use with GeneMapper® *ID* software can be obtained from the Promega web site at: **www.promega.com/resources/software-firmware/str-analysis/genemapper-id-software-panels-and-bin-sets/**

PRODUCT	SIZE	CAT.#
PowerPlex® 4C Matrix Standard	**5 preps**	**DG4800**

Not For Medical Diagnostic Use.

Matrix standards are required for initial setup of the color separation matrix. The matrix standards are sold separately and are available for the Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers (PowerPlex® 4C Matrix Standard). See Section 9.D 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 (9,10). Guidelines for the validation process are published in the *Internal Validation Guide of Autosomal STR Systems for Forensic Laboratories* (11).

The quality of purified DNA, 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 are made to the recommended protocols.

PCR-based STR analysis is subject to contamination by very small amounts of human DNA. Extreme care should be taken to avoid cross-contamination when preparing sample DNA, handling primer pairs, assembling amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (Master Mix, 2800M Control DNA and Primer Pair Mix) are provided in a separate box and should be stored separately from those used following amplification (Allelic Ladder Mix and Internal Lane Standard).

Always include a negative control reaction (i.e., no template) to detect reagent contamination. We highly recommend the use of gloves and aerosol-resistant pipette tips.

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

3.B. Spectral Calibration

Proper spectral calibration is critical to evaluate multicolor systems with the Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers. A matrix must be generated for each individual instrument. The PowerPlex® 4C Matrix Standard (Cat.# DG4800), is required for spectral calibration on the Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers.

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

4. Protocols for DNA Amplification Using the PowerPlex® CS7 System

The PowerPlex® CS7 System is optimized for the GeneAmp® PCR System 9700 thermal cycler. An amplification protocol for the Applied BioSystems ProFlex® PCR System thermal cycler also is 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

We routinely amplify 0.5ng of template DNA in a 25µl reaction volume using the protocols detailed below. Expect to see high peak heights at the smaller loci and relatively 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.

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 thermal cycler or ProFlex® PCR System (Applied Biosystems)
- centrifuge compatible with 96-well plates or reaction tubes
- MicroAmp® optical 96-well reaction plate or 0.2ml MicroAmp® reaction tubes (Applied Biosystems)
- aerosol-resistant pipette tips

Amplification Setup

1. Thaw the PowerPlex® HS 5X Master Mix, PowerPlex® CS7 10X Primer Pair Mix and Water, Amplification Grade, completely.

   Notes:

   a. PowerPlex® HS 5X Master Mix and PowerPlex® CS7 10X Primer Pair Mix are manufactured as a matched set for optimal performance. Do not combine components from kits with different lot numbers (printed on the boxes and Certificates of Analyses). If lots are mixed, locus-to-locus imbalance and variation in signal intensity may occur.

   b. Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 10X Primer Pair Mix or 5X Master Mix after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.

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

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

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

Amplification of >1.0ng 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 2–4 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
Water, Amplification Grade	to a final volume of 25.0µl	×		=	
PowerPlex® HS 5X Master Mix	5.0µl	×		=	
PowerPlex® CS7 10X Primer Pair Mix	2.5µl	×		=	
template DNA (0.5ng)[2,3]	up to 17.5µl				
total reaction volume	**25.0µl**				

[1]Add Water, Amplification Grade, to the tube first, then add PowerPlex® HS Fast 5X Master Mix and PowerPlex® CS7 10X Primer Pair Mix. The template DNA will be added at Step 6.

[2]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. PCR amplification efficiency and quality can be greatly altered by changes in pH (due to added Tris-HCl), available magnesium concentration (due to chelation by EDTA) or other PCR inhibitors, which may be present at low concentrations depending on the source of the template DNA and the extraction procedure used.

[3]Apparent DNA concentrations can differ, depending on the DNA quantification method used (12). We strongly recommend that you perform experiments to determine the optimal DNA amount based on your DNA quantification method and internal validation.

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

Failure to vortex the PCR amplification mix sufficiently can result in poor amplification, peak height imbalance and extra peaks.

6. Add the template DNA (0.5ng) for each sample to the respective well containing PCR amplification mix.
7. For the positive amplification control, vortex the tube of 2800M Control DNA, then dilute an aliquot to 0.5ng in the desired template DNA volume. Add 0.5ng of the diluted DNA to a reaction well containing PCR amplification mix.
8. For the negative amplification control, pipet Water, Amplification Grade, or TE^{-4} buffer instead of template DNA to a reaction well containing PCR amplification mix.
9. Seal or cap the plate, or close the tubes. **Optional:** Briefly centrifuge to bring contents to the bottom of the wells and remove any air bubbles.

4.A. Amplification of Extracted DNA (continued)

Thermal Cycling

This manual contains protocols for use of the PowerPlex® CS7 System with the GeneAmp® PCR System 9700 and Applied Biosystems ProFlex® PCR System thermal cyclers. For information on other thermal cyclers, contact Promega Technical Services by e-mail at: **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 Corporation shows that 10/20 cycles work well for 0.5ng of purified DNA templates. The cycle number can be increased to 10/22 to maximize sensitivity. For higher template amounts or to decrease sensitivity, fewer cycles, such as 10/18 should be evaluated. In-house validation should be performed.

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

   Notes:

   a. When using the ProFlex® PCR System, the ramp rates indicated in the cycling program must be set. The remaining steps must be set to 1.5°C/second.

   b. When using the GeneAmp® PCR System 9700, 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** to adjust the ramp rates at each hold temperature. The ramp speed is set after the thermal cycling run is started. The 'Select Method Options' screen appears. Select **9600** for the ramp speed, and enter the reaction volume.
3. After completion of the thermal cycling protocol, proceed to fragment analysis or store amplified samples at –20°C protected from light.

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

Protocol for the GeneAmp® PCR System 9700 Thermal Cycler	Protocol for the ProFlex® PCR System
96°C for 2 minutes, then:	96°C for 2 minutes, 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 1.5°C/sec to 94°C for 30 seconds ramp 1.2°C/sec to 60°C for 30 seconds ramp 0.4°C/sec 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 20 cycles, then:	ramp 1.5°C/sec to 90°C for 30 seconds ramp 1.2°C/sec to 60°C for 30 seconds ramp 0.4°C/sec to 70°C for 45 seconds for 20 cycles, then:
60°C for 30 minutes	60°C for 30 minutes
4°C soak	4°C soak

4.B. Direct Amplification of DNA from Storage Card Punches

Materials to Be Supplied by the User

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

This section contains a protocol for direct amplification of DNA from storage card punches using the PowerPlex® CS7 System and GeneAmp® PCR System 9700 thermal cycler.

We recommend amplifying one or two 1.2mm punches of a storage card containing buccal cells or one 1.2mm punch of a storage card containing whole blood in a 25µl reaction volume using the protocols detailed below.

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

Lytic storage card sample types include:

- Buccal cells collected on FTA® cards with Whatman EasiCollect™ devices
- Buccal cells collected with swabs transferred to FTA® or Indicating FTA® cards
- Liquid blood (from collection or storage Vacutainer® tubes or finger sticks) spotted onto FTA® cards

Nonlytic storage card sample types include:

- Buccal samples on Bode Buccal DNA Collector™ devices
- Blood and buccal samples on nonlytic storage cards (e.g., S&S 903)

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

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

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

Note: Static may be problematic when adding a punch to a well. For lytic storage card punches, adding PCR amplification mix to the well before adding the punch may help alleviate static problems.

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

Amplification Setup

1. Thaw the PowerPlex® HS 5X Master Mix, PowerPlex® CS7 10X Primer Pair Mix and Water, Amplification Grade, completely.

   Note: Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 10X Primer Pair Mix or 5X Master Mix after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.

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

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

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

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

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	12.5µl	×		=	
PowerPlex® HS 5X Master Mix	5.0µl	×		=	
PowerPlex® CS7 10X Primer Pair Mix	2.5µl	×		=	
5X AmpSolution™ Reagent	5.0µl	×		=	
total reaction volume	**25.0µl**				

[1]Add Water, Amplification Grade, to the tube first, then add PowerPlex® HS 5X Master Mix, PowerPlex® CS7 10X Primer Pair Mix and 5X AmpSolution™ Reagent. For lytic storage card punches, the template DNA will be added at Step 6.

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

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

6. For lytic storage cards, add one or two 1.2mm punches from a card containing a buccal sample or one 1.2mm punch from a card containing whole blood to the appropriate wells of the reaction plate. For nonlytic storage card punches, add the PCR amplification mix to the pretreated punches.

   Note: You can add the lytic storage card punch first, then add the PCR amplification mix.

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:

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

   b. Optimization of the amount of 2800M Control DNA may be required based on thermal cycling conditions and laboratory preferences.

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

   Note: Place the 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 Storage Card Punches (continued)

Thermal Cycling

Amplification and detection instrumentation may vary. You will need to optimize protocols including the number of storage card punches, cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 10/17 cycling works well for a variety of sample types. Buccal samples may require more amplification cycles than blood samples. Cycle number will need to be optimized in each laboratory for each sample type that is amplified.

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

   Note: When using the GeneAmp® PCR System 9700, 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** to adjust the ramp rates at each hold temperature.

   The ramp speed is set after the thermal cycling run is started. The 'Select Method Options' screen appears. Select **9600** for the ramp speed, and enter the reaction volume.

Thermal Cycling Protocol
96°C for 2 minutes, 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

3. After completing the thermal cycling protocol, proceed to fragment analysis or store amplified samples at –20°C protected from light.

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

PCR Optimization

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

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.
2. Depending on your preferred protocol, place one or two 1.2mm storage card punches containing a buccal sample or one 1.2mm storage card punch containing whole blood in each well of a reaction plate. Pretreat nonlytic samples with the PunchSolution™ Kit (Cat.# DC9271).
3. Prepare three identical reaction plates with punches from the same samples.
4. Amplify samples using the thermal cycling protocol provided above, but subject each plate to a different cycle number (10/16, 10/17 and 10/18 cycling).
5. Following amplification, use your laboratory's validated separation and detection protocols to determine the optimal cycle number for the sample type and number of storage card punches.

4.C. Direct Amplification of DNA from Swabs

Materials to Be Supplied by the User

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

This section contains a protocol for amplifying swab extracts using the PowerPlex® CS7 System and GeneAmp® PCR System 9700 thermal cycler.

Pretreat cotton or OmniSwabs™ (QIAGEN) 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 PowerPlex® HS 5X Master Mix, PowerPlex® CS7 10X Primer Pair Mix and Water, Amplification Grade, completely.

   Note: Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 10X Primer Pair Mix or 5X Master Mix after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.
2. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix.

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

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

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

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

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	10.5µl	×		=	
PowerPlex® HS 5X Master Mix	5.0µl	×		=	
PowerPlex® CS7 10X Primer Pair Mix	2.5µl	×		=	
5X AmpSolution™ Reagent	5.0µl	×		=	
swab extract	2.0µl				
total reaction volume	**25.0µl**				

[1]Add Water, Amplification Grade, to the tube first, then add PowerPlex® HS 5X Master Mix, PowerPlex® CS7 10X Primer Pair Mix and 5X AmpSolution™ Reagent. The swab extract will be added at Step 6.

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

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

6. Pipet 2µl 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 an aliquot to 2.5ng/µl, and add 2µl to a reaction well containing 23µl of PCR amplification mix.

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

8. For the negative amplification control, pipet 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 or cap the plate, or close the tubes.

   Optional: Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

Thermal Cycling

Amplification and detection instrumentation may vary. You will need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 10/18 cycling works well for a variety of sample types. Cycle number will need to be optimized in each laboratory for each sample type that is amplified (see below).

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

   Note: When using the GeneAmp® PCR System 9700, 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** to adjust the ramp rates at each hold temperature.

   The ramp speed is set after the thermal cycling run is started. The 'Select Method Options' screen appears. Select **9600** for the ramp speed, and enter the reaction volume.

Thermal Cycling Protocol
96°C for 2 minutes, 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

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

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

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 (10/17, 10/18 and 10/19 cycling).

   Note: This recommendation is for 2µl of swab extract. Additional cycle number testing may be required.
4. Following amplification, use your laboratory's validated separation and detection protocols to determine the optimal cycle number for the sample type.

5. Instrument Setup and Sample Preparation

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

Materials to Be Supplied by the User

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

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

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

Sample Preparation

1. Prepare a loading cocktail by combining and mixing internal lane standard (ILS) and formamide as follows:

   [(0.5µl ILS) × (# samples)] + [(9.5µl 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.

2. Vortex for 10–15 seconds to mix.

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

4. Add 1µl of amplified sample (or 1µl of Allelic Ladder Mix) 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. To modify the injection time or injection voltage in the run module, select **Instrument Protocol** from the Library menu in the data collection software. If peak heights are higher than desired, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program by 2–4 cycles to achieve the desired signal intensity.

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

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

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

Instrument Preparation

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

1. Open the 3500 Data Collection Software. The Dashboard screen will launch (Figure 1). Ensure that the Consumables Information and Maintenance Notifications are acceptable.

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

Figure 1. The Dashboard.

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

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

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

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

   Assign a descriptive protocol name.

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

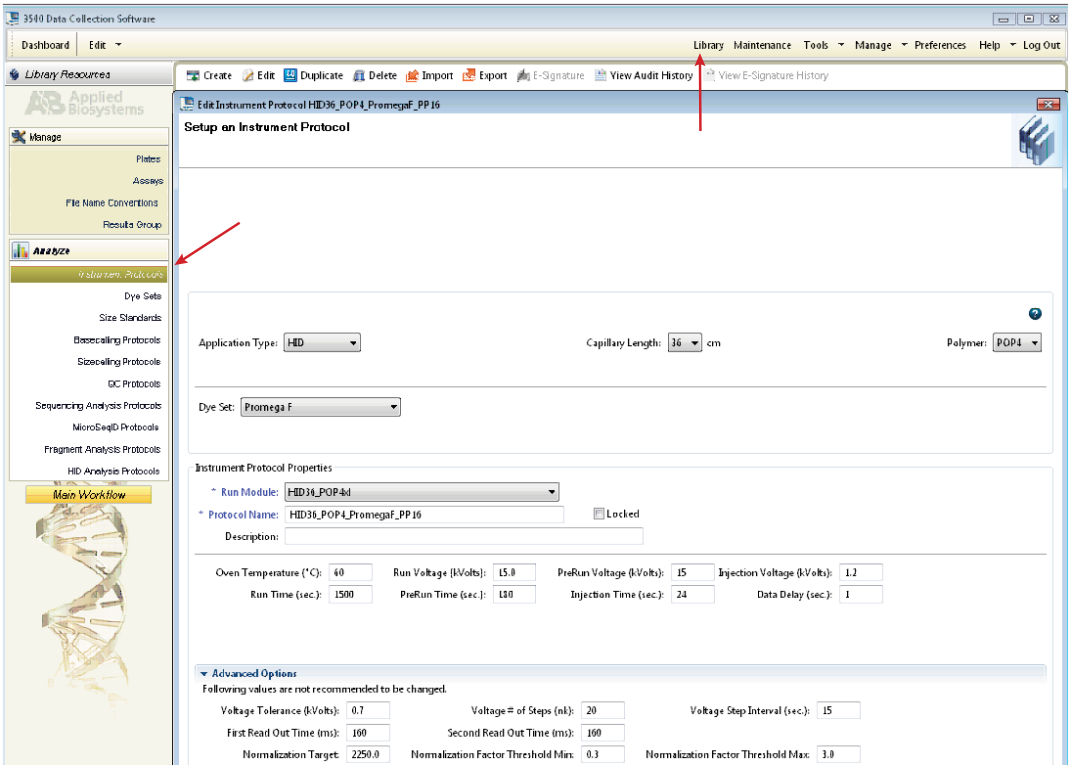

Figure 2. The Create New Instrument Protocol window.

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

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

   Assign the size standard the name "PPLX_ILS600" or another appropriate name. Choose **Red** as the Dye Color. The fragments in the size standard are 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases. See Figure 3.

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

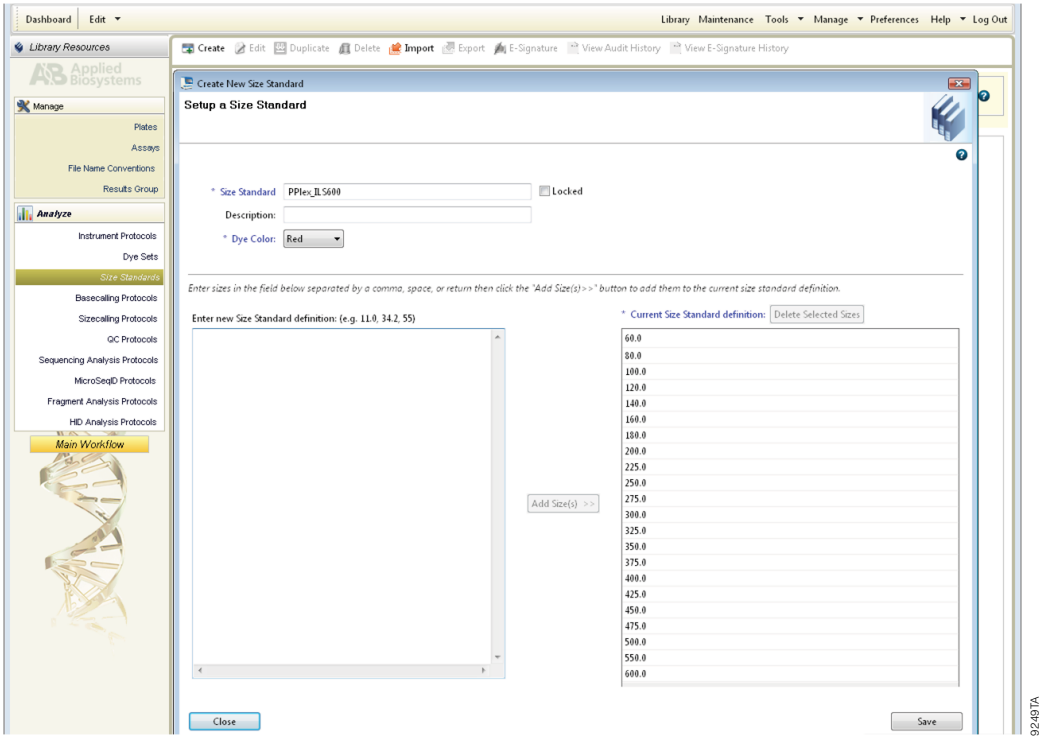

Figure 3. The 'Create New Size Standard' window.

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

    Assign a descriptive protocol name. Select the size standard created in Step 3. The settings for the QC protocol should be based on the internally validated conditions for the PowerPlex® CS7 System on the Applied Biosystems® 3500 or 3500xL Genetic Analyzer. Figure 4 shows one option for these settings.

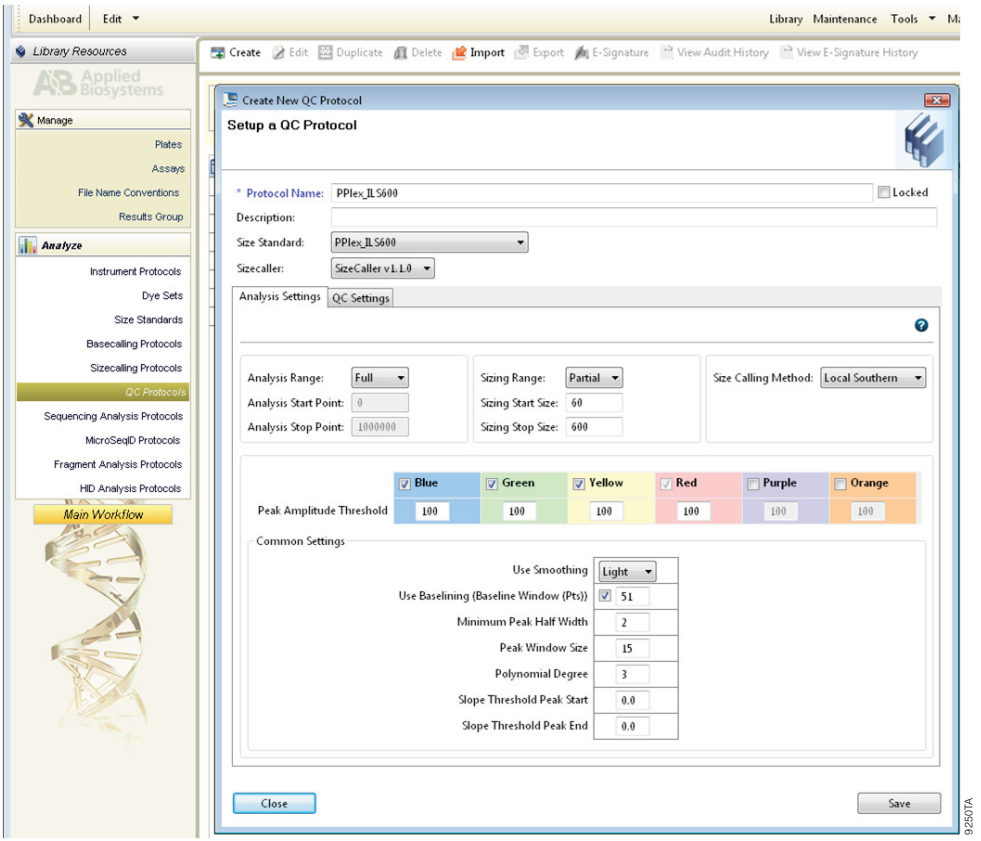

Figure 4. The 'Create New QC Protocol' window.

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

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

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

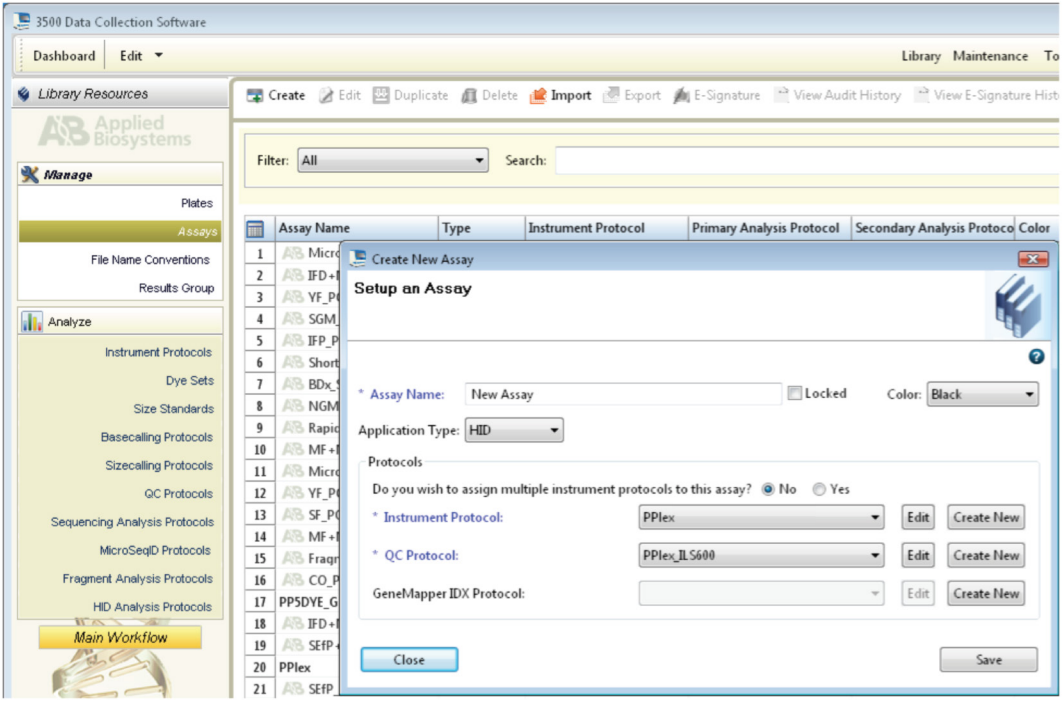

Figure 5. The 'Create New Assay' window.

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

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

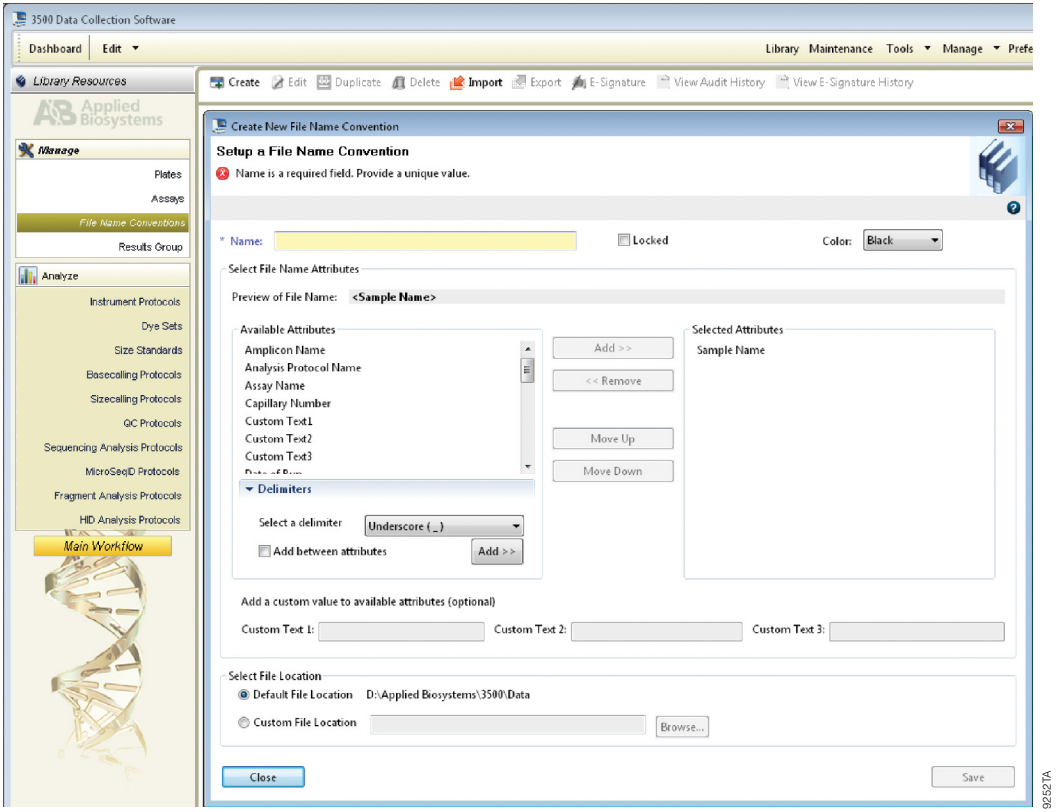

Figure 6. The 'Create New File Name Convention' window.

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

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

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

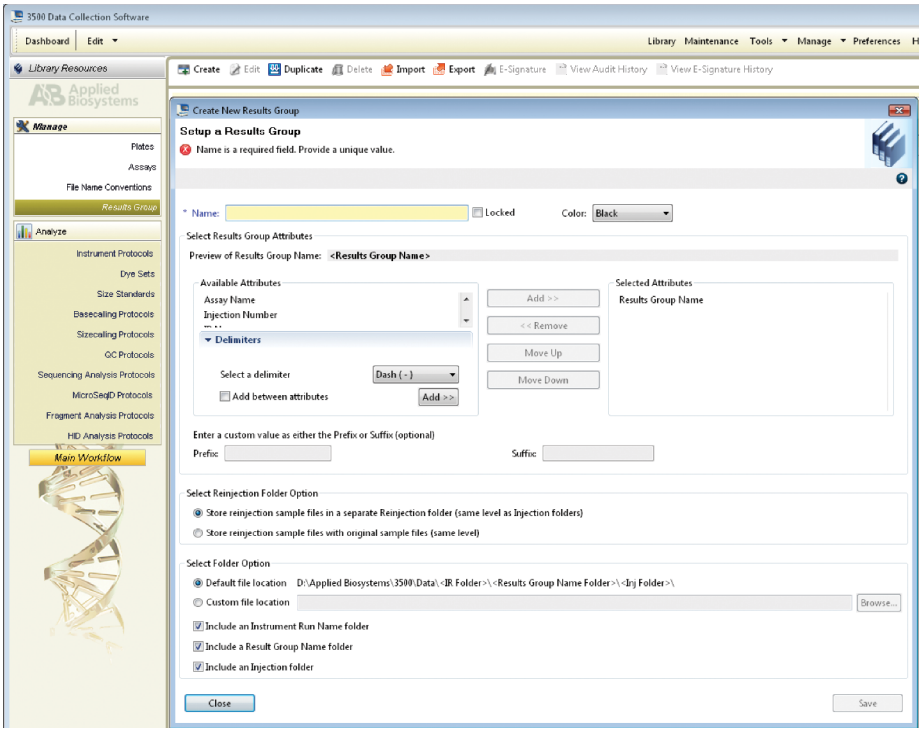

Figure 7. The 'Create New Results Group' window.

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

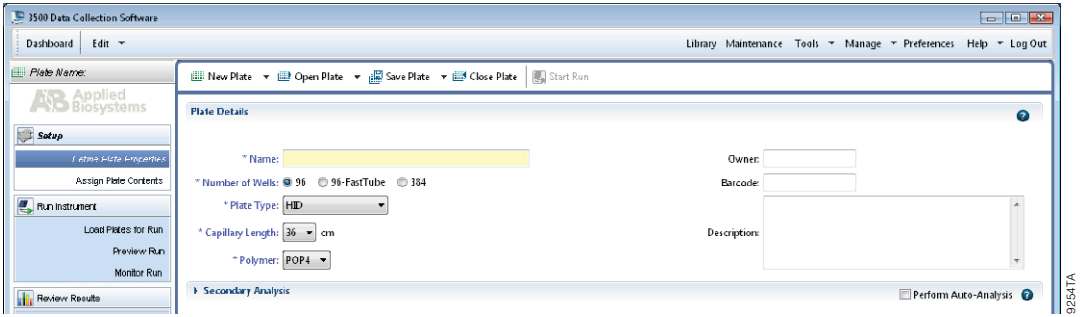

Figure 8. Defining plate properties.

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

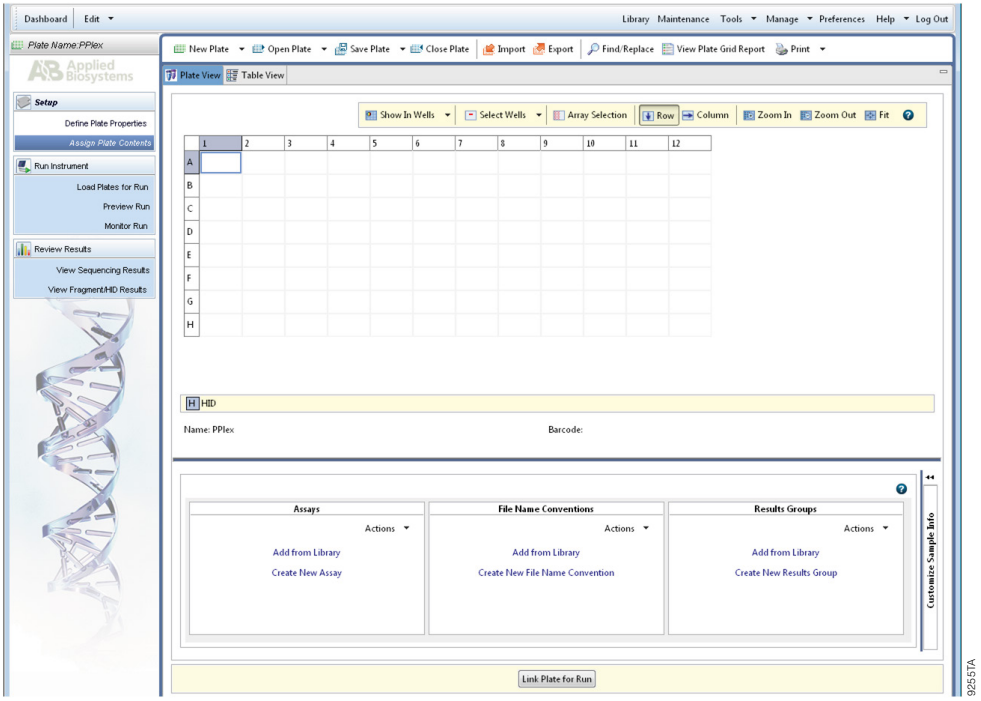

Figure 9. Assigning plate contents.

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

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

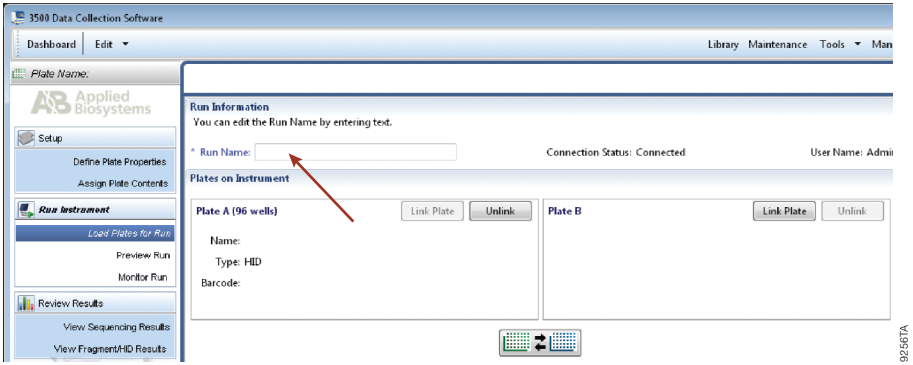

Figure 10. Assigning a run name.

5.B. Detection of Amplified Fragments Using 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, ice-water bath or freezer plate block
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3130 capillary array, 36cm
- POP-4® polymer for the Applied Biosystems® 3130/3130*xl*
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate and septa (Applied Biosystems)
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

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

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

Sample Preparation

1. Thaw the Internal Lane Standard.

   Note: Centrifuge tubes 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 (ILS) and formamide as follows:

   [(0.5µl of ILS) × (# samples)] + [(9.5µl of 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.

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 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 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, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program by 2–4 cycles to achieve the desired signal intensity.

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

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

Instrument Preparation

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

Analyze samples as described in the user's manual for the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software with the following exceptions.

1. In the Module Manager, select **New**. Select **Regular** in the 'Type' drop-down list, and select **HIDFragmentAnalysis36_POP4** in the 'Template' drop-down list. Confirm that the injection time is 5 seconds and the injection voltage is 3kV. Lengthen the run time to 1,800 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 fields, and select **OK**.

   Note: If autoanalysis of sample data is desired, refer to the instrument user's manual for instructions.
4. In the GeneMapper® plate record, enter sample names in the appropriate cells. Scroll to the right. In the Results Group 1 column, select the desired results group. In the Instrument Protocol 1 column, select the protocol you created in Step 2. Be sure this information is present for each row that contains a sample name. Select **OK**.

   Note: To create a new results group, select **New** in the drop-down menu in the Results Group column. Select the 'General' tab, and enter a name. Select the 'Analysis' tab, and select **GeneMapper—Generic** in the 'Analysis' type drop-down list.
5. Place samples in the instrument, and close the instrument doors.
6. In the spectral viewer, confirm that dye set F is active, and set the correct active spectral 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, select the plate graphic that corresponds to the plate on the autosampler that contains your amplified samples.

9. When the plate record is linked to the plate, the plate graphic changes 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.

6. Data Analysis

6.A. Importing PowerPlex® CS7 Panels and Bins Text Files with GeneMapper® *ID* Software, Version 3.2

To facilitate analysis of data generated with the PowerPlex® CS7 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 the PowerPlex® CS7 System go to: **www.promega.com/resources/software-firmware/str-analysis/genemapper-id-software-panels-and-bin-sets/**
2. Enter your contact information, and select **GeneMapper ID** and the control DNA that you use. Select **Submit**.
3. Save the PowerPlex_CS7_Panels_vX.x.txt and PowerPlex_CS7_Bins_vX.x.txt files, where "X.x" refers to the most recent version of the panels and bins text files, to a known location on your computer.

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**, and then **Panel Manager**.
3. Highlight the **Panel Manager** icon in the upper left navigation pane.
4. Select **File**, and then **Import Panels**.
5. Navigate to the panels text file downloaded in the Getting Started section above. Select the file, and then **Import**.
6. In the navigation pane, highlight the Promega 16 HS CS7 panels folder that you just imported in Step 5.
7. Select **File**, and then **Import Bin Set**.
8. Navigate to the bins text file downloaded in the Getting Started section above. Select the file, and then **Import**.
9. At the bottom of the 'Panel Manager' window, select **OK**. This saves the panels and bins text files and closes the window automatically.

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

1. Select **Tools**, and then **GeneMapper Manager**.
2. Select the 'Size Standard' tab.
3. Select **New**.
4. Select **Basic or Advanced** (Figure 11). The type of analysis method selected must match the type of analysis method created earlier. Select **OK**.

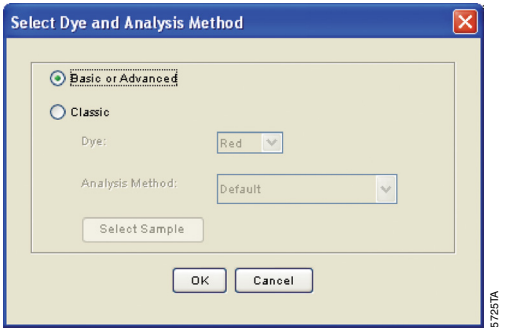

Figure 11. The 'Select Dye and Analysis Method' window.

5. Enter a detailed name, such as “ILS 600 Advanced”, in the Size Standard Editor (Figure 12).

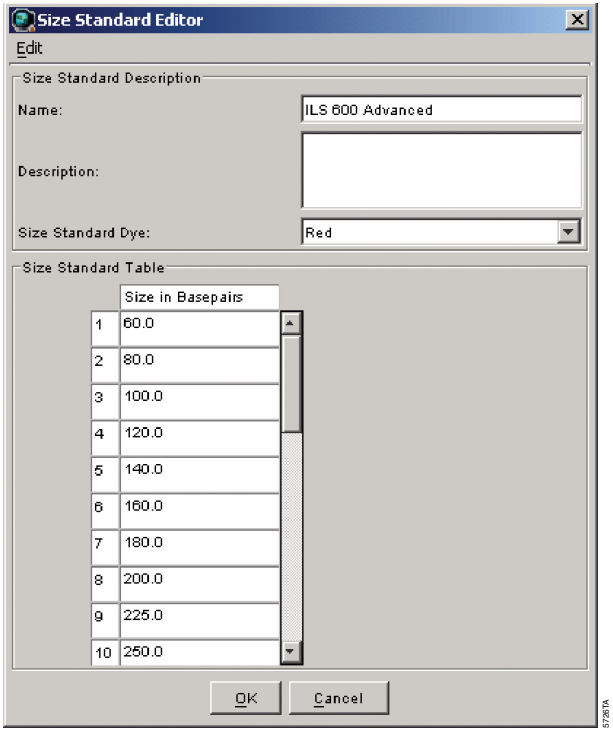

Figure 12. The Size Standard Editor

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.C, Figure 23.

   Note: Definition and detection of the 600bp fragment is optional.
8. Select **OK**.

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

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

1. Select **Tools**, and then **GeneMapper Manager**.
2. Select the ‘Analysis Methods’ tab.
3. Select **New**, and a new analysis method dialog box will open.

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

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. In the Analysis Method Editor, enter a descriptive name for the analysis method, such as "PowerPlexCS7_20%filter".

6. Select the 'Allele' tab (Figure 13).

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 13 for proper filtering of peaks when using the PowerPlex® CS7 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*".

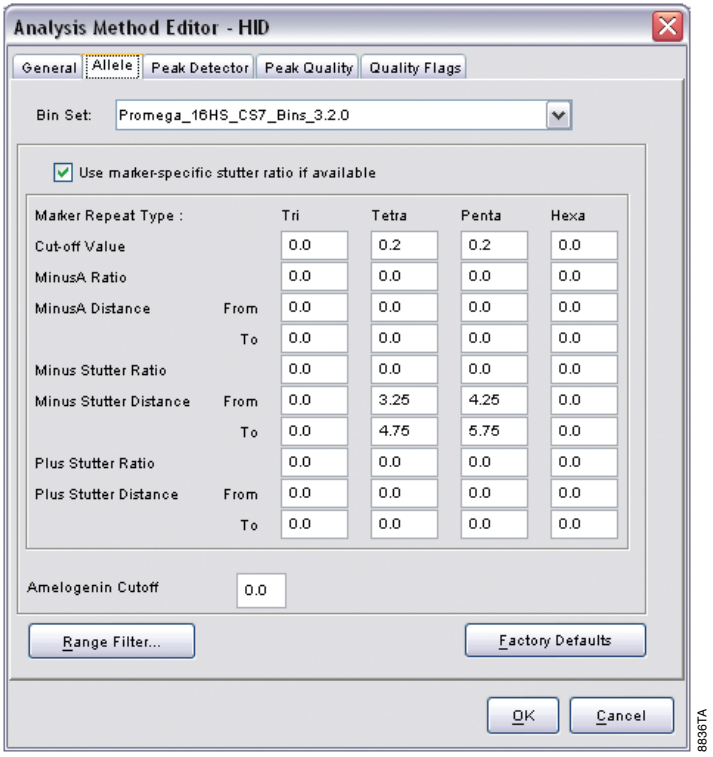

Figure 13. The GeneMapper® *ID* 'Allele' tab with settings for using a 20% peak filter.

10. Select the 'Peak Detector' tab. We recommend the settings shown in Figure 14.

    Notes:

    a. 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.

    b. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Values for peak amplitude thresholds should be determined by individual laboratories.

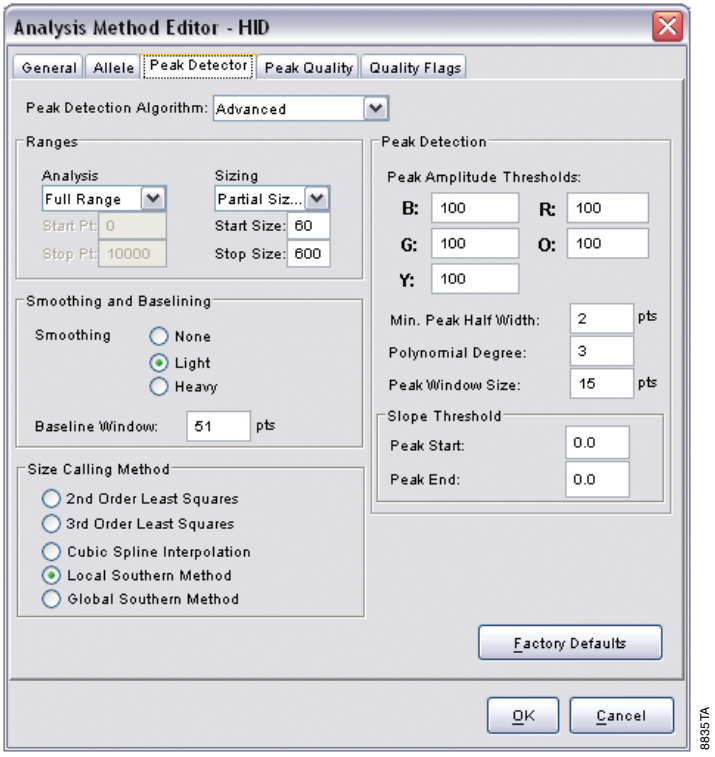

Figure 14. The GeneMapper® *ID* 'Peak Detector' tab.

11. Select the 'Peak Quality' tab to change the settings for peak quality.

12. Select the 'Quality Flags' tab to change these settings.

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

13. Select **OK** to save your settings.

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

Processing Data for Databasing or Paternity Samples

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

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

These instructions are intended as a guide to start analyzing data in GeneMapper® *ID* software. They are not intended as a comprehensive guide for using the GeneMapper® *ID* software. We recommend that users contact Applied Biosystems for training on the software. These instructions loosely follow the Applied Biosystems GeneMapper® *ID* software tutorial, pages 5–11.

1. Select **Tools**, and 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. In the Analysis Method Editor, enter a descriptive name for the analysis method, such as "PowerPlexCS7 advanced".
6. Select the 'Allele' tab (Figure 15).
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.

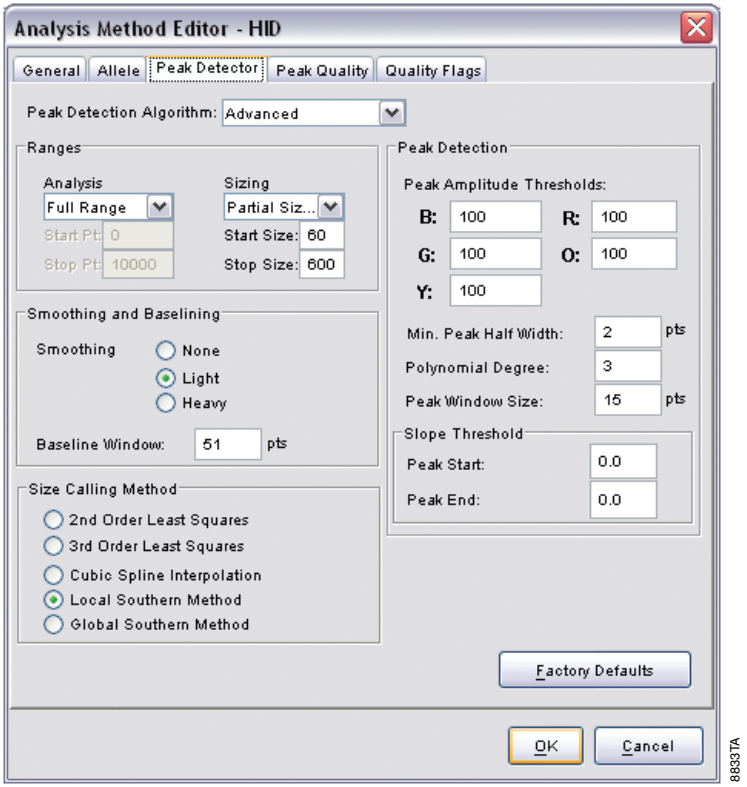

Figure 15. The GeneMapper® *ID* 'Allele' tab.

9. Enter the values shown in Figure 15 for proper filtering of stutter peaks when using the PowerPlex® CS7 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.

10. Select the 'Peak Detector' tab. We recommend the settings shown in Figure 16.

    Notes:

    a. 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.

    b. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Values for peak amplitude thresholds should be determined by individual laboratories.

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

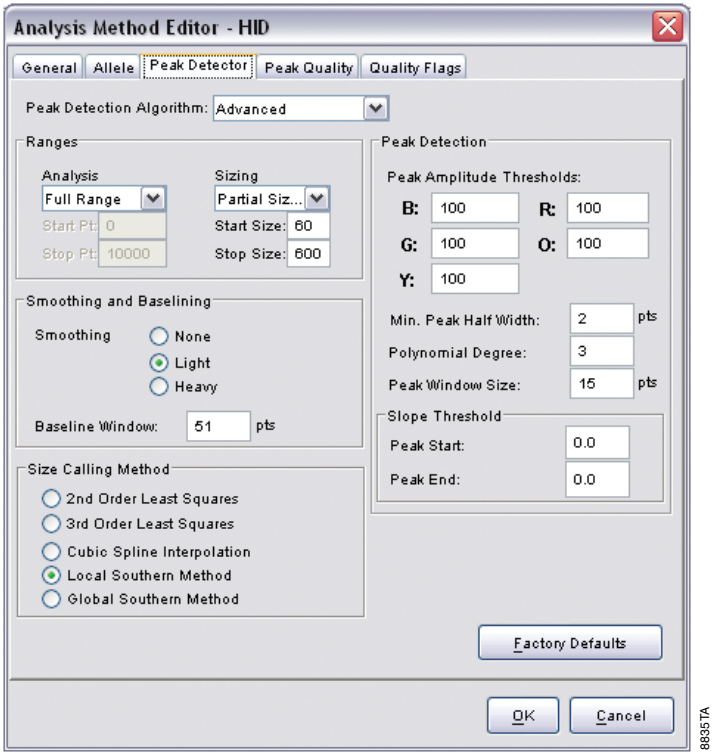

Figure 16. The GeneMapper® *ID* 'Peak Detector' tab.

11. Select the 'Peak Quality' tab. You may change the settings for peak quality.
12. Select the 'Quality Flags' tab. You may change these settings.
    Note: For Steps 11 and 12, see the GeneMapper® *ID* user's manual for more information.
13. Select **OK** to save your settings.

Processing Data for Casework Samples

1. Select **File**, and then **New Project**.
2. Select **Edit**, and then **Add Samples to Project**.
3. Browse to the location of the run files. Highlight desired files, and then select **Add to list** followed by **Add**.
4. In the 'Sample Type' column, use the drop-down menu to select **Ladder**, **Sample**, **Positive Control** or **Negative Control** as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated as **Ladder** in the 'Sample Type' column for proper genotyping.

5. In the 'Analysis Method' column, select the analysis method created previously in this section.
6. In the 'Panel' column, select the panels text file that was imported in Section 6.A.
7. In the 'Size Standard' column, select the size standard that was created in Section 6.B.
8. Select **Analyze** (green arrow button) to start data analysis.

6.E. 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. Compare the 2800M Control DNA allelic repeat sizes with the locus-specific allelic ladder. The expected 2800M Control DNA allele designations for each locus are listed in Table 4 (Section 9.A).

6.F. Results

Representative results of the PowerPlex® CS7 System are shown in Figure 17. The PowerPlex® CS7 Allelic Ladder Mix is shown in Figure 18.

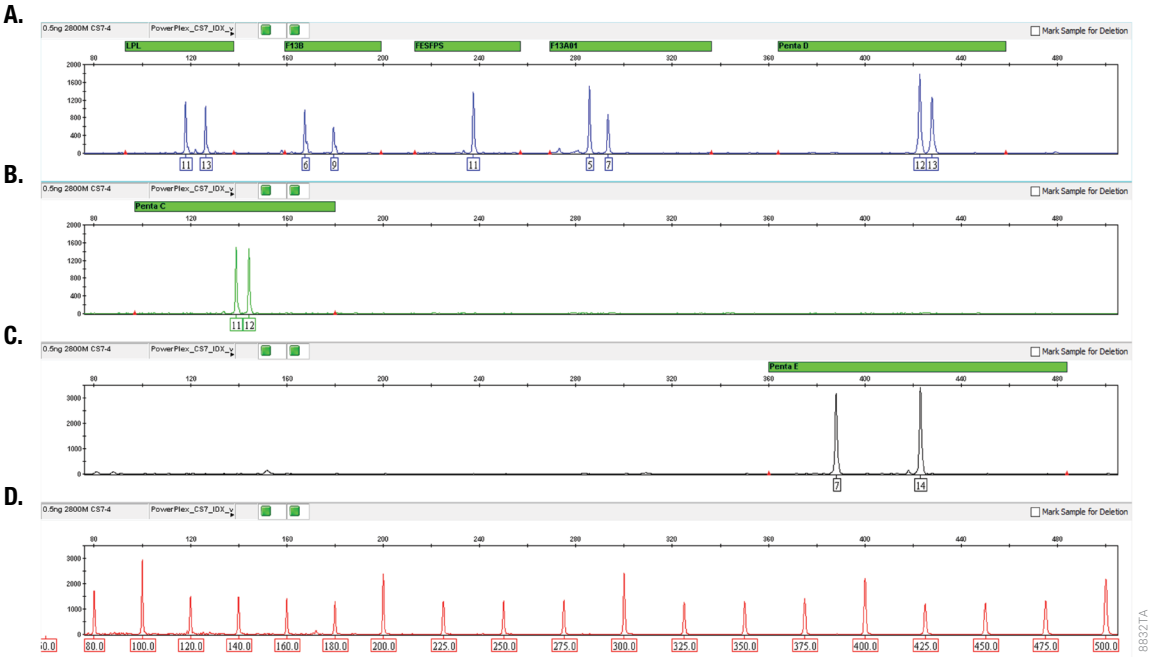

Figure 17. The PowerPlex® CS7 System. A single-source template DNA (0.5ng) was amplified using the PowerPlex® CS7 System. Amplification products were mixed with Internal Lane Standard 600 and analyzed with an Applied Biosystems® 3500xL Genetic Analyzer using a 1.2kV, 24-second injection. Results were analyzed using GeneMapper® *ID*-X software, version 1.4, and PowerPlex® CS7 panels and bins text files. **Panel A.** An electropherogram showing the peaks of the fluorescein-labeled loci: LPL, F13B, FESFPS, F13A01 and Penta D. **Panel B.** An electropherogram showing the peaks of the JOE-labeled locus: Penta C. **Panel C.** An electropherogram showing the peaks of the TMR-labeled locus: Penta E. **Panel D**. An electropherogram showing the 80–500bp fragments of the Internal Lane Standard 600.

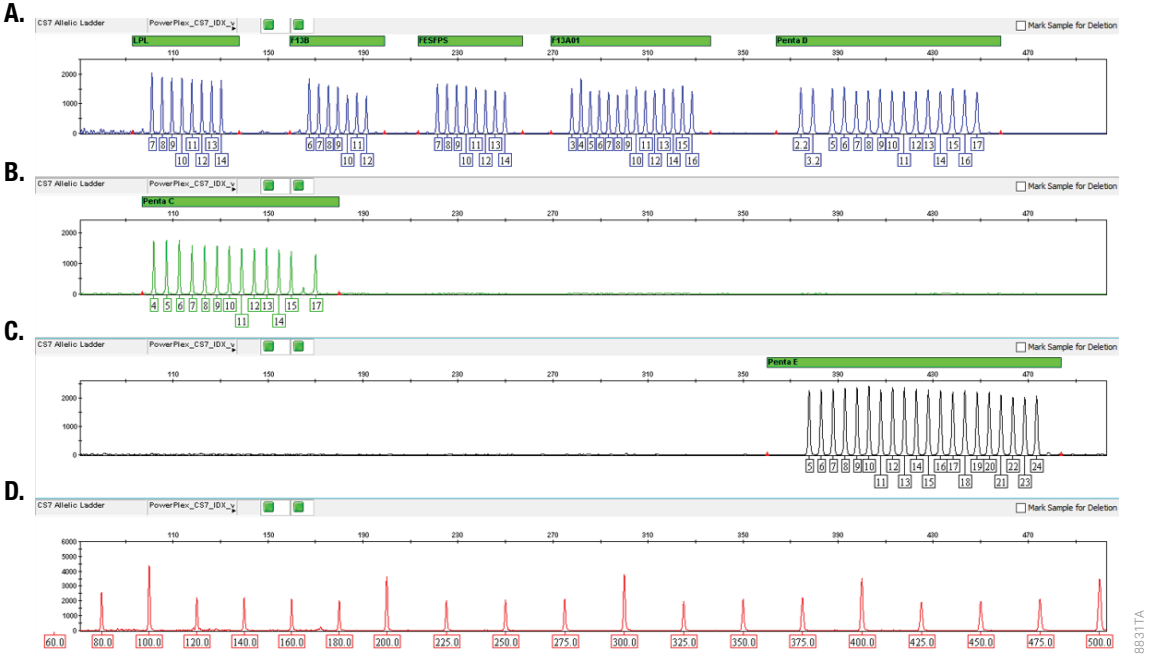

Figure 18. The PowerPlex® CS7 Allelic Ladder Mix. The PowerPlex® CS7 Allelic Ladder Mix was analyzed with an Applied Biosystems® 3500xL Genetic Analyzer using a 1.2kV, 24-second injection. The sample file was analyzed with the GeneMapper® *ID*-X software, version 1.4, and PowerPlex® CS7 panels and bins 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. **Panel D**. An electropherogram showing the 80–500bp fragments of the Internal Lane Standard 600.

Artifacts and Stutter

Stutter products are a common amplification artifact associated with STR analysis (13,14). 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.

In addition to stutter peaks, other artifact peaks can be observed at some of the PowerPlex® CS7 loci. Low-level products can be seen at n–1 at LPL and Penta C, at n–9 and n+1 at F13B, and at n–12 to n–13 at FESPS and F13A01. When the amplified peaks are particularly intense, one or more extra peaks can be seen occasionally in the fluorescein channel at 254bp, 273bp, 301bp, 357bp, 379bp, 429bp or 479bp.

7. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **genetic@promega.com**

7.A. Amplification and Fragment Detection

This section provides information about general amplification and detection. For questions about direct amplification, see Sections 7.B and 7.C.

Symptoms	Causes and Comments
Faint or absent allele peaks	The Master Mix was not vortexed well before use. Vortex the Master Mix for 15 seconds before dispensing into the PCR amplification mix.
	Primer concentration was too low. Use the recommended primer concentration. Vortex the Primer Pair Mix for 15 seconds before use.
	An air bubble formed at the bottom of the reaction tube. Use a pipette to remove the air bubble or centrifuge the reactions briefly before thermal cycling.
	Thermal cycler, plate or tube problems. Review the thermal cycling protocols. We have not tested reaction tubes, plates or thermal cyclers that are not listed. Calibrate the thermal cycler heating block if necessary.
	Repeat sample preparation using fresh formamide. Long-term storage of amplified sample in formamide can result in loss of signal.
	Poor capillary electrophoresis injection (ILS peaks also affected). Re-inject the sample.
	Laser is starting to fail. Check laser power.
	Poor-quality formamide was used. Use only Hi-Di™ formamide when analyzing samples.
Faint or absent peaks for the positive control reaction	Improper storage of the 2800M Control DNA. Store the 2800M Control DNA at +2°C to +10°C. Make sure that the 2800M Control DNA is stored at +2°C to +10°C for at least 24 hours before use; do not refreeze. Vortex before use.

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels	Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips and change gloves regularly.
	Do not store the PCR amplification mix for a prolonged period. Add the mix to the wells of the reaction plate as soon as the mix is prepared. Add the DNA as soon as possible to each well and follow immediately by thermal cycling.
	Samples were not denatured completely. Heat-denature samples for the recommended time, and cool on crushed ice, a freezer plate block or in an ice-water bath immediately prior to capillary electrophoresis. Do not cool the samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing.
	Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis. Appearance of "shadow" peaks migrating in front of the main peaks, especially if the shadow peaks are separated by the same distance as the main peaks in a heterozygote, can indicate the presence of double-stranded DNA due to incomplete denaturation or post-injection re-annealing.
	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 fluorescein and JOE channels. Use autoclaved deionized water; change vials and wash buffer reservoir.
	Incorrect spectral was active when analyzing samples with the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer. Rerun samples, and confirm that the Promega 4C spectral calibration is set for dye set F. See instructions for instrument preparation in Section 5.B.

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 is applied to the samples. • Perform a new spectral calibration and rerun the samples. • Confirm that the correct spectral was used for the sample run. • Instrument sensitivities can vary. Optimize the injection conditions. Refer to the instrument user manual. • Reboot the Applied Biosystems® 3500 or 3500xL Genetic Analyzer and the instrument's computer. Repeat the spectral calibration. Do not allow borrowing when running the spectral calibration on the Applied Biosystems® 3500 or 3500xL Genetic Analyzer.
	Dye blob artifacts. The signal strength of certain dye blob artifacts increases with storage of the amplification plate at 4°C, sometimes in as short a time period as overnight but more commonly when left at 4°C for a few days. We recommend storing amplification products at –20°C.
	An incorrect internal lane standard was used. Use the size standard provided in the kit.
	The CE polymer was beyond its expiration date, or polymer was stored at room temperature for more than one week.
	Maintain instrumentation on a 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.
	Be sure the allelic ladder and samples are from the same instrument run.
	Migration of samples changed slightly over the course of a CE run with many samples. This may be due to changes in temperature or the CE capillary over time. Use a different injection of allelic ladder to determine sizes.
	Poor injection of allelic ladder. Include more than one ladder per instrument run.
	Internal size standard was not assigned correctly. Evaluate the sizing labels on the ILS and correct if necessary.

Symptoms	Causes and Comments
Peak height imbalance	Miscellaneous balance problems. At the first use, thaw the Primer Pair Mix and Master Mix completely. Vortex the Primer Pair Mix and Master Mix for 15 seconds before use; do not centrifuge the Primer Pair Mix or Master Mix after mixing. Calibrate thermal cyclers and pipettes routinely.
	PCR amplification mix was not mixed well. Vortex the PCR amplification mix for 5–10 seconds before dispensing into the reaction tubes or plate.
	Tubes of Master Mix and Primer Mix from different kit lots were used. The Master Mix and Primer Mix are manufactured as a matched set for optimal performance. If kit lots are mixed, locus-to-locus imbalance and variation in signal intensity may occur.

7.B. Direct Amplification of DNA from Storage Card Punches

The following information is specific to direct amplification of DNA from storage card punches. For additional information about general amplification and detection, see Section 7.A.

Symptoms	Causes and Comments
Faint or absent allele peaks	For direct amplification reactions that require the addition of AmpSolution™ Reagent, its omission can result in inhibition of the amplification reaction. Review the amplification setup and confirm whether or not AmpSolution™ Reagent is required for your direct amplification reactions.
	DNA was not accessible on nonlytic material. Pretreat nonlytic materials with PunchSolution™ Reagent to ensure that DNA is liberated from cellular proteins.
	Poor sample deposition. Shedding and collection of donor cells was variable. Increase cycle number.
	Poor sample transfer to storage card or variable sampling from the storage card. Take punches from a different portion of the card. Increasing cycle number can improve low peak heights.
	Too much sample in the reaction can result in inhibition, decreasing intensity of peaks (especially larger amplicons). Be sure to use the recommended number of punches. Follow the manufacturer's recommendations when depositing sample onto the storage card.

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

Symptoms	Causes and Comments
Faint or absent allele peaks (continued)	Amplification was inhibited when using more than one storage card punch with blood. Use only one 1.2mm storage card punch with blood.
	The reaction volume was too low. Decreasing the reaction volume from that recommended in the protocol may result in suboptimal performance. Use the recommended number of punches for the reaction volume used.
	Active PunchSolution™ Reagent carried over into the amplification reaction when using nonlytic storage 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 was used to pretreat nonlytic storage card punches. Thaw the PunchSolution™ Reagent at +2°C to +10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.
	Consider using PunchSolution™ Reagent with lytic storage card punches.
Faint or absent peaks for the positive control reaction	If the positive control reaction failed to amplify, check to make sure that the correct amount of 2800M Control DNA was added to the reaction. Due to the reduced cycle number, it is necessary to increase the mass of 2800M Control DNA to obtain a profile. Use the recommended amount of 2800M Control DNA per amplification reaction. This mass of DNA should be reduced if the cycle number is increased and increased if the cycle number is decreased. Increase or decrease by twofold the mass of 2800M Control DNA for every one-cycle decrease or increase, respectively. We do not recommend including blank punches in the 2800M Control DNA reaction.

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels	Punch was contaminated with DNA from another sample. Perform punches on a blank card between samples to minimize potential for carryover between samples.
	Amplification of processed punches with high amounts of DNA can result in artifact peaks due to overamplification, resulting in saturating signal on the CE instrument. Be sure to use the recommended number of punches. Use of a larger punch size or a smaller reaction volume may result in overamplification and signal saturation. If the signal is saturated, repeat the amplification with a smaller punch, a larger reaction volume or reduced cycle number.
	Amplification of excess template for a given cycle number can result in overloading of the capillary upon electrokinetic injection. The presence of excess DNA in the capillary makes it difficult to maintain the DNA in a denatured single-stranded state. Some single-stranded DNA renatures and becomes double-stranded. Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis and appears as "shadow" peaks migrating in front of the main peaks. If this occurs at a heterozygous locus, it is sometimes possible to see two "shadow" peaks that differ in size from one another by approximately the same distance as the single-stranded alleles.
	Artifacts of STR amplification. Direct amplification of high amounts of template can result in a higher number of artifact peaks. Use the recommended punch size and number of punches. Optimize the cycle number. See Results section for additional information on stutter and artifacts.
	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´A residue. • Be sure to perform the recommended extension step at 60°C after thermal cycling. • Decrease cycle number. • Increase the final extension time.

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

Symptoms	Causes and Comments
Peak height imbalance	Excessive amount of DNA. Amplification of high amounts of template can result in an imbalance, with smaller loci showing more product than larger loci. • Be sure to use the recommended number of punches. Follow the manufacturer's recommendation when depositing sample onto the card. • Decrease cycle number.
	The cycle number was too high. Decrease the cycle number by one cycle and repeat the amplification.
	For direct amplification reactions that require the addition of AmpSolution™ Reagent, its omission can result in inhibition of the amplification reaction. Review the amplification setup and confirm whether or not AmpSolution™ Reagent is required for your direct amplification reactions.
	The reaction volume was too low. Decreasing the reaction volume can result in suboptimal performance due to inhibitors present in lytic cards and PunchSolution™ Reagent.
	DNA was not accessible on nonlytic material. Small loci may amplify preferentially, with large loci dropping out. Pretreat nonlytic materials with PunchSolution™ Reagent to ensure that DNA is released from cellular proteins.
	Active PunchSolution™ Reagent carried over into the amplification reaction with nonlytic card punches. Larger loci are most susceptible to carryover and will drop out before the smaller loci. • Ensure that the heat block reached 70°C and samples were incubated for 30 minutes or until wells are dry. Incubation for shorter time periods may result in incomplete inactivation of the PunchSolution™ Reagent. • We recommend treating one 1.2mm nonlytic card punch with 10µl of PunchSolution™ Reagent and using one punch per 25µl or 12.5µl amplification reaction. Reducing the PunchSolution™ Reagent volume may improve results for reactions with reduced amplification volumes. Optimization and validation are required.

Symptoms	Causes and Comments
Peak height imbalance (continued)	Inactive PunchSolution™ Reagent was used to pretreat nonlytic storage card punches. Thaw the PunchSolution™ Reagent at +2°C to +10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.
Extreme variability in sample-to-sample peak heights	There can be significant individual-to-individual variability in the number of cells on a card resulting in peak height variability between samples. The PunchSolution™ Kit maximizes the recovery of amplifiable DNA from nonlytic storage card punches 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 after pretreatment using the SwabSolution™ Kit. 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°C to +10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.
	Active SwabSolution™ Reagent carried over into the amplification reaction. Ensure that the heat block reached 70°C (90°C if using a 2.2ml, Square-Well Deep Well Plate) and samples were incubated for the full 30 minutes. Incubation for shorter time periods may result in incomplete reagent inactivation. Do not use an incubator to incubate tubes or plates; heat transfer is inefficient and will result in poor performance. Use only a heat block to maintain efficient heat transfer. We have tested 60-minute incubation times and observed no difference in performance compared to a 30-minute incubation.

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

Symptoms	Causes and Comments
Faint or absent allele peaks (continued)	For direct amplification reactions that require the addition of AmpSolution™ Reagent, its omission can result in inhibition of the amplification reaction. Review the amplification setup and confirm whether or not AmpSolution™ Reagent is required for your direct amplification reactions.
	DNA was not accessible on nonlytic material. Pretreat swabs with SwabSolution™ Reagent to ensure that DNA is liberated from cellular proteins.
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. Use the recommended amount of 2800M Control DNA per amplification reaction. This mass of DNA should be reduced if the cycle number is increased and increased if the cycle number is decreased. Increase or decrease by twofold the mass of 2800M Control DNA for every one-cycle decrease or increase, respectively.
Extra peaks visible in one or all color channels	Swab extract was contaminated. Assemble a reaction containing the swab extract prepared from a blank swab, or assemble a reaction where the SwabSolution™ Reagent is processed and incubated as a blank without a swab.
	Artifacts of STR amplification. Amplification of swab extracts with high DNA concentrations can result in artifact peaks due to overamplification, resulting in saturated signal on the CE instrument. We recommend 2µl of swab extract per reaction. Using more than 2µl may result in overamplification and signal saturation. If signal is saturated, repeat amplification with less swab extract or reduced cycle number.

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels (continued)	Amplification of excess template for a given cycle number resulted in overloading of the capillary upon electrokinetic injection. Excess DNA in the capillary is difficult to maintain in a denatured single-stranded state. Some single-stranded DNA renatures and becomes double-stranded. Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis and appears as "shadow" peaks migrating in front of the main peaks. If this occurs at a heterozygous locus it is possible to observe the presence of two "shadow" peaks that differ in size by approximately the same distance as the single-stranded alleles.
	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´A residue. • Be sure to perform the recommended extension step at 60°C after thermal cycling. • Use 2µl of swab extract in an amplification reaction. A larger volume of swab extract may contain more than the recommended amount of DNA template, resulting in incomplete adenylation. • Decrease cycle number. • Increase the final extension time.
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 from swab extracts into the amplification reaction. Larger loci are most susceptible to reagent carryover and will drop out before the smaller loci. Ensure that the heat block reached 70°C (90°C if using 2.2ml, Square-Well Deep Well Plates) and samples were incubated for the full 30 minutes. Incubation for shorter time periods may result in incomplete reagent inactivation. Do not use an incubator to incubate tubes or plates; heat transfer is inefficient and will result in poor performance. Use only a heat block to maintain efficient heat transfer.

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

Symptoms	Causes and Comments
Peak height imbalance (continued)	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°C to +10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.
	DNA was not accessible on nonlytic material. Small loci may amplify preferentially, with large loci dropping out. Pretreat swabs with SwabSolution™ Reagent to ensure that DNA is liberated from cellular proteins.
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, quantify the DNA using a fluorescence-based double-stranded DNA quantification method or qPCR-based quantification method. The quantification values can be used to normalize input template amounts to minimize variation in signal intensity.

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.
	To analyze samples with GeneMapper® *ID* software, at least one allelic ladder must be defined per folder of sample files being analyzed in the project.
	Run was too short and larger peaks in ILS were not captured. Not all ILS peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Rerun samples using a longer run time.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.

Symptoms	Causes and Comments
Off-ladder alleles	An allelic ladder from a different run than the samples was used. Reanalyze samples using an allelic ladder from the same run.
	The GeneMapper® *ID* software requires that the allelic ladder be imported from the same folder as the sample. Be sure that the allelic ladder is in the same folder as the sample. Create a new project and re-analyze as described in Section 6.G or 6.H.
	Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file that corresponds to the STR system used for amplification.
	The allelic ladder was not identified as an allelic ladder in the Sample Type column.
	The wrong analysis type was chosen for the analysis method. Be sure to use the HID analysis type.
	The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
Size standard not called correctly	Starting data point was incorrect for the partial range chosen in Section 6.G or 6.H. Adjust the starting data point in the analysis method. Alternatively, use a full range for the analysis.
	Extra peaks in advanced mode size standard. Open the Size Match Editor. Highlight the extra peak, select **Edit** and select **Delete Size Label**. Select **Auto Adjust Sizes**.
	Run was too short, and larger peaks in ILS were not captured. Not all ILS peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Rerun samples using a longer run time.
Peaks in size standard missing	If peaks are low-quality, redefine the size standard for the sample to skip these peaks.
	An incorrect size standard was used.
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.

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

Symptoms	Causes and Comments
No alleles called, but no error message appears	Panels text file was not selected for sample. In the Panel column, select the appropriate panels text file for the STR system that was used.
	No size standard was selected. In the Size Standard column, be sure to select the appropriate size standard.
	Size standard was not correctly defined, or size peaks were missing. Redefine size standard to include only peaks present in your sample. Terminating analysis early or using short run times will cause larger ladder peaks to be missing. This will cause your sizing quality to be flagged as red, and no allele sizes will be called.
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 'Allele' tab, and select an appropriate bins text file.
	The wrong bins text file was chosen in the analysis method 'Allele' tab. Be sure to choose the appropriate bins text file, as shown in Figure 15.
Significantly raised baseline	Poor spectral calibration. Perform a new spectral calibration and rerun the samples.
	Use of Classic mode analysis method. Use of Classic mode analysis on samples can result in baselines with more noise than those analyzed using the Basic or Advanced mode analysis method. Advanced mode analysis methods and size standards are recommended.
	Incorrect spectral was active when analyzing samples with the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer. Rerun samples, and confirm that the Promega 4C spectral calibration is set for dye set F. See instructions for instrument preparation in Section 5.B.
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 reimport files in a different order.

Symptoms	Causes and Comments
Allelic ladder peaks 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.

8. References

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9. Appendix

9.A. Advantages of STR Typing

The loci included in the PowerPlex® CS7 System are listed in Tables 4 and 5. Table 6 lists the PowerPlex® CS7 System alleles revealed in 2800M Control DNA.

Table 4. The PowerPlex® CS7 System Locus-Specific Information.

STR Locus	Label	Chromosomal Location	GenBank® Locus and Locus Definition	Repeat Sequence[1] 5´→ 3´
LPL	FL	8p22	HUMLIPOL, Human lipoprotein lipase gene	AAAT
F13B	FL	1q31–q32.1	HUMBFXIII, Human factor XIII b subunit gene	AAAT
FESFPS	FL	15q25–qter	HUMFESFPS, Human c fes/fps proto-oncogene	AAAT
F13A01	FL	6p24–p25	HUMF13A01, Human coagulation factor XIII a subunit gene	AAAG
Penta D	FL	21q	NA	AAAGA
Penta C	JOE	9p13	NA	AAAGC
Penta E	TMR	15q	NA	AAAGA

[1]The August 1997 report (15,16) 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".

TMR = carboxy-tetramethylrhodamine

FL = fluorescein

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

NA = not applicable

9.A. Advantages of STR Typing (continued)

Table 5. The PowerPlex® CS7 System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components[1,2] (bases)	Repeat Numbers of Allelic Ladder Components[3]
LPL	FL	105–133	7–14
F13B	FL	169–193	6–12
FESFPS	FL	222–250	7–14
F13A01	FL	279–331	3–16
Penta D	FL	373–446	2.2, 3.2, 5–17
Penta C	JOE	104–169	4–15, 17
Penta E	TMR	376–471	5–24

[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]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: **https://strbase.nist.gov/var_tab.htm**

[4]Amelogenin is not an STR.

Table 6. The PowerPlex® CS7 System Allele Determinations for the 2800M Control DNA.

STR Locus	2800M
LPL	11, 13
F13B	6, 9
FESFPS	11, 11
F13A01	5, 7
Penta D	12, 13
Penta C	11, 12
Penta E	7, 14

9.B. 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 19). Each fragment is labeled with carboxy-X-rhodamine (CXR) and can be detected separately (as a fourth color) in the presence of PowerPlex® CS7-amplified material. The ILS 600 is designed for use in each CE injection to increase precision in analyses when using the PowerPlex® CS7 System. The protocols to prepare and use of this internal lane standard are provided in Section 5.

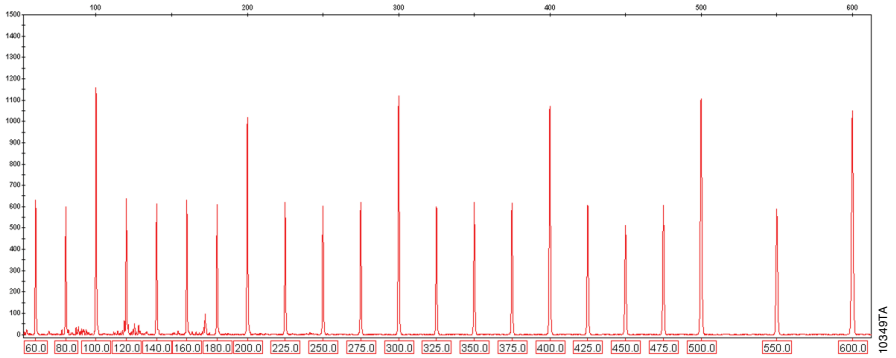

Figure 19. Internal Lane Standard 600. An electropherogram showing the Internal Lane Standard 600 fragments.

9.C. Composition of Buffers and Solutions

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.

Promega

9.D. Related Products

Product	Size	Cat.#
PowerPlex® 4C Matrix Standard*	5 preps	DG4800
Internal Lane Standard 600	150µl	DG1071
Water, Amplification Grade*	5 × 1,250µl	DW0991
2800M Control DNA (10ng/µl)*	25µl	DD7101
2800M Control DNA (0.25ng/µl)*	500µl	DD7251
PunchSolution™ Kit*	100 preps	DC9271
SwabSolution™ Kit*	100 preps	DC8271
5X AmpSolution™ Reagent*	500µl	DM1231

*Not for Medical Diagnostic Use.

10. Summary of Changes

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

1. Removed mention of ABI PRISM® Genetic Analyzers.
2. Added ProFlex® PCR System and removed GeneAmp® PCR System 9600 thermal cycler.
3. Updated Section 6.J.
4. Removed original Section 9.B.
5. Revised Section 9.D.
6. Removed disclaimers.
7. Incorporated other general updates.
8. Moved to a new template.