PowerPlex® ESX 17 Fast System for Use on the Spectrum CE System Technical Manual

Instructions for Use of Products DC1710 and DC1711

TMD079 | 12/22

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All technical literature is available at: **www.promega.com/protocols/**

Visit the web site to verify that you are using the most current version of this Technical Manual.

E-mail Promega Technical Services if you have questions on use of this system: **genetic@promega.com**

1 Description

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

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

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

The PowerPlex® ESX 17 Fast System and all system components are manufactured in accordance with ISO 18385:2016. All necessary materials are provided to amplify STR regions of human genomic DNA, including a hot-start thermostable DNA polymerase, which is a component of the PowerPlex® ESI/ESX Fast 5X Master Mix. This manual contains a protocol for use of the PowerPlex® ESX 17 Fast System with the GeneAmp® PCR System 9700, Veriti® 96-Well Thermal Cycler and ProFlex® PCR System, in addition to a protocol to separate amplified products and detect separated material on the Spectrum CE System. A protocol to operate the Spectrum CE System is available separately: see the *Spectrum CE System Operating Manual* #TMD052. Amplification and detection instrumentation may vary. You may need to optimize protocols, including amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. In-house validation should be performed.

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

2 Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
PowerPlex® ESX 17 Fast System	100 reactions	DC1711

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

Pre-Amplification Components Box

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

Post-Amplification Components Box

- 50µl PowerPlex® ESX 17 Fast Allelic Ladder Mix
- 200µl WEN Internal Lane Standard 500 ESS

PRODUCT	SIZE	CAT.#
PowerPlex® ESX 17 Fast System	400 reactions	DC1710

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

Pre-Amplification Components Box

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

Post-Amplification Components Box

- 4 × 50µl PowerPlex® ESX 17 Fast Allelic Ladder Mix
- 2 × 200µl WEN Internal Lane Standard 500 ESS

The PowerPlex® ESX 17 Fast Allelic Ladder Mix is provided in a separate, sealed bag for shipping. After opening, move the PowerPlex® ESX 17 Fast Allelic Ladder Mix to the post-amplification box. Water, Amplification Grade, is provided in a separate, sealed bag for shipping. After opening, move the Water, Amplification Grade, to the pre-amplification box.

Storage Conditions:

Upon receipt, store all components except the 2800M Control DNA at –30°C to –10°C in a nonfrost-free freezer. Store the 2800M Control DNA at +2°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. After the first use, store the WEN Internal Lane Standard (WEN ILS) 500 ESS at +2°C to +10°C, protected from light; do not refreeze. The PowerPlex® ESX 17 Fast 10X Primer Pair Mix, PowerPlex® ESX 17 Fast Allelic Ladder Mix and WEN ILS 500 ESS are light-sensitive and must be stored in the dark. We strongly recommend that pre-amplification and post-amplification reagents be stored and used separately with different pipettes, tube racks, etc.

Optional: The PowerPlex® ESX 17 Fast System components can be stored for up to 1 year at +2°C to +10°C without loss of activity.

Available Separately

PRODUCT	SIZE	CAT.#
PunchSolution™ Kit	100 preps	DC9271
SwabSolution™ Kit	100 preps	DC8271
5X AmpSolution™ Reagent	500µl	DM1231
GeneMarker®HID Software for Spectrum CE Systems, Local	1 seat	CE3001
GeneMarker®HID Software for Spectrum CE Systems, Network	1 seat	CE3010
GeneMarker®HID Software for Spectrum CE Systems, Client	1 seat	CE3011

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

The proper panels, bins and stutter text files for use with GeneMapper® *ID*-X software are available for download at: **www.promega.com/PowerPlexPanels**

The proper panel .xml file for use with GeneMarker®HID Software for Spectrum CE Systems (GMHID-Spectrum) is preloaded in GMHID-Spectrum or downloadable at **www.promega.com/PowerPlexPanels**

The PowerPlex® 5C Matrix Standard (Cat.# DG4850) is required for initial setup of the color separation matrix (see Section 3.2).

3 Before You Begin

3.1 Precautions

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

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

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

3.2 Spectral Calibration

Proper spectral calibration is critical to evaluate multicolor systems with the Spectrum CE System. A matrix must be generated for each individual instrument.

For protocol and additional information about matrix generation and spectral calibration, see the *PowerPlex® Matrix Standards for Use on the Spectrum CE System Technical Manual* #TMD068. This manual is available at: **www.promega.com/protocols/**

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

The PowerPlex® ESX 17 Fast System was developed for amplification of extracted DNA and direct-amplification samples. Slight protocol variations are recommended for optimal performance with each template source. Protocols for amplification in a 25µl reaction volume using extracted DNA (Section 4.1), lytic and nonlytic storage card punches (Section 4.2) and swabs (Section 4.3) are included in the following amplification sections. Protocols for amplification in a 12.5µl reaction volume using lytic and nonlytic storage card punches and swabs are included in Sections 11.3 and 11.4, respectively.

The PowerPlex® ESX 17 Fast System is compatible with the GeneAmp® PCR System 9700 with a gold-plated silver or silver sample block, the Veriti® 96-Well Thermal Cycler and the ProFlex® PCR System. This system has not been tested with the Veriti® 96-Well Fast Thermal Cycler.

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

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 9.

4.1 Amplification of Extracted DNA in a 25µl Reaction Volume

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

Materials to be Supplied by the User

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

Amplification Setup

1. Thaw the PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESX 17 Fast 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 1 to a clean tube.

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® ESI/ESX Fast 5X Master Mix	5.0µl	×		=	
PowerPlex® ESX 17 Fast 10X Primer Pair Mix	2.5µl	×		=	
template DNA (0.5ng)[2,3,4]	up to 17.5µl				
total reaction volume	25.0µl				

[1]Add Water, Amplification Grade, to the tube first, then add PowerPlex® ESI/ESX Fast 5X Master Mix and PowerPlex® ESX 17 Fast 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 (14). We strongly recommend that you perform experiments to determine the optimal DNA amount based on your DNA quantification method.

[4]The PowerPlex® ESX 17 Fast System is optimized and balanced for 0.5ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your internal validation and may be different.

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

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

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

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

Note: The PowerPlex® ESX 17 Fast System is optimized and balanced for 0.5ng of DNA template. The amount of DNA template used in your laboratory should be based on the results of your internal validation and may be different.

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

8. For the negative amplification control, pipet Water, Amplification Grade, or TE^{-4} buffer instead of template DNA into a reaction well containing PCR amplification mix.
9. Seal or cap the plate, or close the tubes.
   Optional: Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

Thermal Cycling

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

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

Notes:

a. When using the ProFlex® PCR System, use the 9700 Simulation Mode as the ramp speed.

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

c. When using the GeneAmp® PCR System 9700, the program must be run with Max Mode as the ramp speed. This requires a gold-plated silver or silver sample block. The ramp speed is set after the thermal cycling run is started. When the 'Select Method Options' screen appears, select **Max** for the ramp speed and enter the reaction volume.

Thermal Cycling Protocol
96°C for 1 minute, then:
96°C for 5 seconds 60°C for 35 seconds 72°C for 5 seconds for 30 cycles, then:
60°C for 2 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.

4.2 Direct Amplification of DNA from Storage Card Punches in a 25µl Reaction Volume

Materials to be Supplied by the User

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

This section contains a protocol for direct amplification of DNA from storage card punches in a 25µl reaction volume using the PowerPlex® ESX 17 Fast System and GeneAmp® PCR System 9700, Veriti® 96-Well Thermal Cycler or ProFlex® PCR System. A protocol for direct amplification of DNA from storage card punches in a 12.5µl reaction volume is provided in Section 11.3.

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

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

Lytic card sample types include:

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

Nonlytic card sample types include:

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

Pretreat nonlytic sample types with the PunchSolution™ Kit (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 card punches, adding PCR amplification mix to the well before adding the punch may help alleviate static problems. For nonlytic card punches, adding PunchSolution™ Reagent to the well before adding the punch during pretreatment may help alleviate static problems.

Amplification Setup

1. Thaw the PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESX 17 Fast 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. Vortex the 5X AmpSolution™ Reagent for 10–15 seconds.

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

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

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

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

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

PCR Amplification Mix Component[1]	Volume per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	12.5µl	×		=	
PowerPlex® ESI/ESX Fast 5X Master Mix	5.0µl	×		=	
PowerPlex® ESX 17 Fast 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, and then add PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESX 17 Fast 10X Primer Pair Mix and 5X AmpSolution™ Reagent. For lytic card punches, the template DNA will be added at Step 7.

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

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

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

7. For 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 card punches, add the PCR amplification mix to the PunchSolution™ Reagent-treated punches.

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

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

   Notes:

   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 depending on thermal cycling conditions and laboratory preferences. Typically, 10ng of 2800M Control DNA is sufficient to provide a robust profile using the cycle numbers recommended here. A one-cycle reduction in cycle number will require a twofold increase in mass of DNA template to generate similar signal intensity. Similarly, a one-cycle increase in cycle number will require a twofold reduction in the amount of 2800M Control DNA to avoid signal saturation.

9. Reserve a well containing PCR amplification mix as a negative amplification control.

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

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

Thermal Cycling

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

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

   Notes:

   a. When using the ProFlex® PCR System, use the 9700 Simulation Mode as the ramp speed.

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

   c. When using the GeneAmp® PCR System 9700, the program must be run with Max Mode as the ramp speed. This requires a gold-plated silver or silver sample block. The ramp speed is set after the thermal cycling run is started. When the 'Select Method Options' screen appears, select **Max** for the ramp speed and enter the reaction volume.

Thermal Cycling Protocol
96°C for 1 minute, then:
96°C for 5 seconds 60°C for 35 seconds 72°C for 5 seconds for 26 cycles, then:
60°C for 2 minutes
4°C soak

3. After completion of the thermal cycling protocol, proceed with 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 buccal cells or one 1.2mm punch of a storage card containing whole blood into each well of a reaction plate. Be sure to 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 (25, 26 and 27 cycles).
5. Following amplification, use your laboratory's validated separation and detection protocols to determine the optimal cycle number for the sample type and number of storage card punches.

4.3 Direct Amplification of DNA from Swabs in a 25µl Reaction Volume

Materials to be Supplied by the User

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

This section contains a protocol for amplifying DNA from swab extracts in a 25µl reaction volume using the PowerPlex® ESX 17 Fast System and GeneAmp® PCR System 9700, Veriti® 96-Well Thermal Cycler or ProFlex® PCR System. A protocol for direct amplification of DNA from swab extracts in a 12.5µl reaction volume is provided in Section 11.4.

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

Amplification Setup

1. Thaw the PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESX 17 Fast 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. Vortex the 5X AmpSolution™ Reagent for 10–15 seconds.

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

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

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

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

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

PCR Amplification Mix Component[1]	Volume per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	10.5µl	×		=	
PowerPlex® ESI/ESX Fast 5X Master Mix	5.0µl	×		=	
PowerPlex® ESX 17 Fast 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, and then add PowerPlex® ESI/ESX Fast 5X Master Mix, PowerPlex® ESX 17 Fast 10X Primer Pair Mix and 5X AmpSolution™ Reagent. The swab extract will be added at Step 7.

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

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

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

7. Pipet 2µl of swab extract for each sample into the appropriate well of the reaction plate or tube.

8. For the positive amplification control, vortex the tube of 2800M Control DNA, then dilute an aliquot to 5ng/µl. Add 2µl (10ng) to a reaction well containing 23µl of PCR amplification mix.

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

9. For the negative amplification control, pipet 2µl of 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.

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

Thermal Cycling

Amplification and detection instrumentation may vary. You will need to optimize protocols including the amount of template DNA, cycle number (24–27 cycles), injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 26 cycles works well for a variety of swab sample types. Cycle number will need to be optimized in each laboratory for each sample type that is amplified (see below).

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

Notes:

a. When using the ProFlex® PCR System, use the 9700 Simulation Mode as the ramp speed.

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

c. When using the GeneAmp® PCR System 9700, the program must be run with Max Mode as the ramp speed. This requires a gold-plated silver or silver sample block. The ramp speed is set after the thermal cycling run is started. When the 'Select Method Options' screen appears, select **Max** for the ramp speed and enter the reaction volume.

Thermal Cycling Protocol
96°C for 1 minute, then:
96°C for 5 seconds 60°C for 35 seconds 72°C for 5 seconds for 26 cycles, then:
60°C for 2 minutes
4°C soak

3. After completion of the thermal cycling protocol, proceed with 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 and instrumentation.

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.
2. Prepare three identical reaction plates with aliquots of the same swab extracts.
3. Amplify samples using the thermal cycling protocol provided above, but subject each plate to a different cycle number (25, 26 and 27 cycles).

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

5 Detection of Amplified Fragments Using the Spectrum CE System

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
- Spectrum Capillary Array, 8-Capillary (Cat.# CE2008)
- Spectrum Polymer4, 384 Wells (Cat.# CE2048) or
  Spectrum Polymer4, 960 Wells (Cat.# CE2040)
- Spectrum Buffer (Cat.# CE2001)
- Spectrum Cathode Septa Mat (Cat.# CE2002)
- Septa Mat, 96-Well (Cat.# CE2696) or equivalent Applied Biosystems septa mat
- Spectrum Plate Base & Retainer, 96-Well (Cat.# CE5004)
- MicroAmp® optical 96-well plate (or equivalent; Applied Biosystems)
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

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

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

Note: Wear gloves when handling consumables and sample cartridges.

5.1 Sample Preparation

1. Prepare a loading cocktail by combining and mixing internal lane standard (ILS) and Hi-Di™ 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 adjusted to change the intensity of the size standard peaks based on laboratory preferences.

2. Vortex for 10–15 seconds to mix.
3. Pipet 10µl of formamide/internal lane standard mix into each well of the 96-well plate.
4. Add 1µl of amplified sample (or 1µl of Allelic Ladder Mix) to each well. Cover wells with appropriate septa.

   Notes:

   a. Instrument detection limits vary; therefore, injection time or the amount of sample mixed with loading cocktail may need to be increased or decreased. To modify the injection time, refer to the *Spectrum CE System Operating Manual* #TMD052. If peak heights are higher than desired, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program to achieve the desired signal intensity.

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

   c. Include an injection of allelic ladder every 2 injections (16 samples).

5. Centrifuge the plate briefly to remove air bubbles from the wells.
6. Denature samples at 95°C for 3 minutes, and then immediately chill on crushed ice or a freezer plate block or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

5.2 Instrument Preparation

Refer to the *Spectrum CE System Operating Manual* #TMD052 for the instrument maintenance schedule and instructions to install the capillary array, anode buffer, cathode buffer and polymer pouch. The appropriate spectral calibration must be performed and accepted on the instrument prior to use for samples. Refer to the *PowerPlex® Matrix Standards for Use on the Spectrum CE System Technical Manual* #TMD068 for instructions on how to perform a spectral calibration.

Open the Spectrum Control Software (SCS). Upon launching the SCS, the 'Home' screen will be displayed (Figure 1). This screen provides access to the four workflow menus (Home, Review Plates, Maintenance and Settings) as well as status indicators. The screen is divided into three sections: Header, Task Pane and Status Bar.

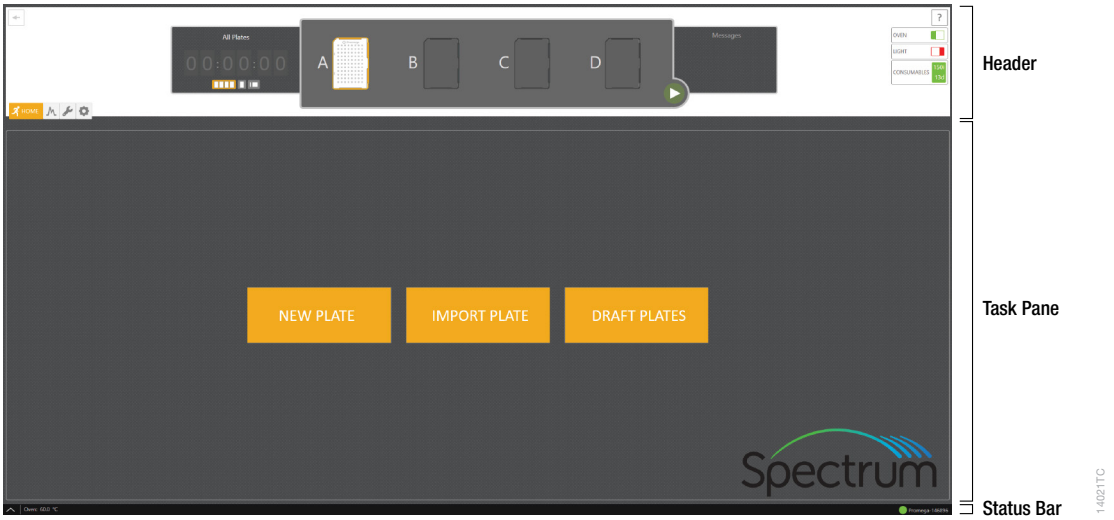


Figure 1. Spectrum Control Software 'Home' screen.

The Header (Figure 2) is fixed and remains available to the user throughout all navigation processes. This area contains Status Indicators, Message Center, Start Button, Run Time Indicator and Workflow Menu. The header also shows the status of the plate positions within the Plate Drawer.

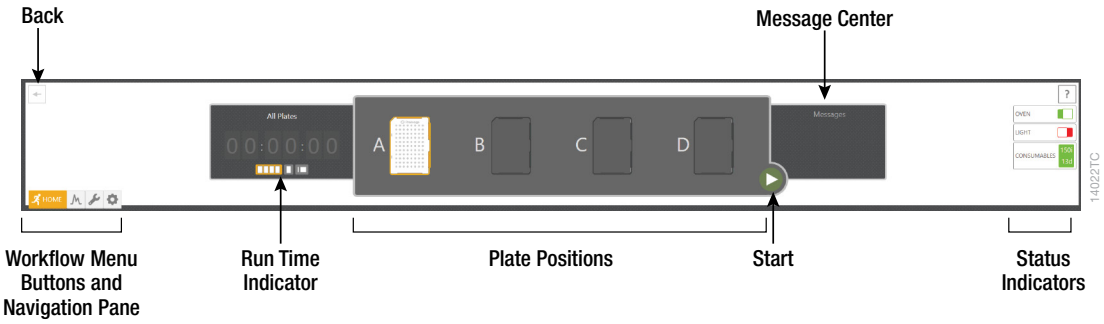


Figure 2. Header.

Each button in the Workflow Menu provides access to a list of workflow-specific functions. Workflow Buttons and the Navigation Pane are highlighted to indicate the user's current location within the SCS.

Icon	Button Name	Function
	Back	Navigates to the previous screen
	Start/stop	Starts or stops all currently scheduled plates (enabled when plates are linked for run and sufficient consumables are available)
	Help	Opens the PDF of the *Spectrum CE System Operating Manual*
HOME	Home	Displays Home Menu
	Review Plates	Displays Review Plates Menu
	Maintenance	Displays Maintenance Menu
	Settings	Displays Settings Menu

The SCS contains several indicators in the header. Each indicator provides information about a specific function or component. Refer to the *Spectrum CE System Operating Manual* #TMD052 for more information.

Before starting a run, ensure all consumables are installed and in sufficient supply. For best results, use unexpired reagents that are within the recommended use range. Refer to the 'Consumables' screen (Figure 3) to determine if any consumables need to be replaced. To access the 'Consumables' screen, select the **Consumables** status indicator in the header on the SCS screen (Figure 2). The 'Consumables' screen (Figure 3) displays information for the four RFID-tagged consumables on the instrument: Polymer, Capillary Array and Anode and Cathode Buffers.

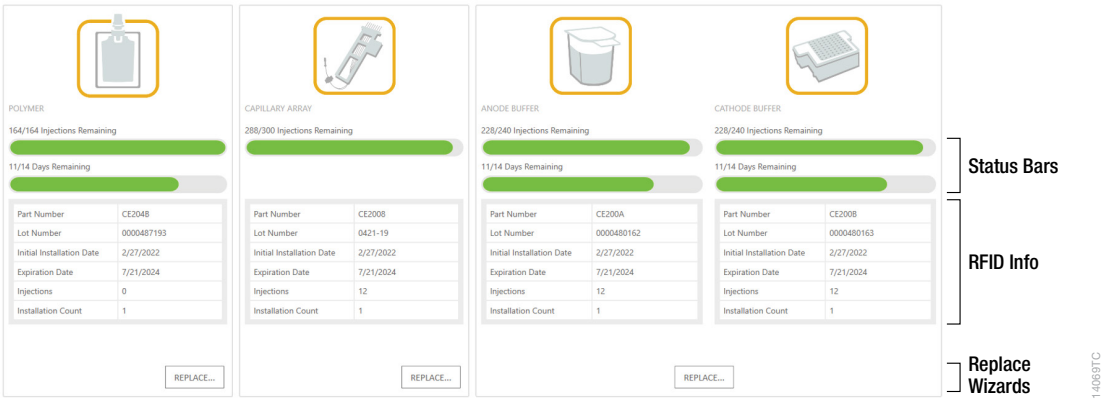


Figure 3. 'Consumables' screen.

The following information is tracked through these tags:

• Part Number

• Lot Number

• Initial Installation Date

• Expiration Date

• Injections

• Installation Count

Refer to the *Spectrum CE System Operating Manual* #TMD052 for more details and information on installing consumables.

Select the **Oven Heater Indicator** in the header (Figure 2) to start preheating the oven to 60°C. The oven temperature will be displayed in the status bar at the bottom of the screen and the indicator will change from red to green when the oven preheating is complete.

Note: We recommend you preheat the oven for at least 30 minutes prior to starting a run. The oven will automatically turn off after 2 hours if a run is not started. Inspect the fluid block, tubing, polymer syringe and polymer pouch for bubbles, and perform a bubble purge if necessary.

5.3 Create a Plate Record

On the 'Home' screen, there are three options displayed for plate setup: New Plate, Import Plate and Draft Plates (Figure 1).

Create a plate record for each plate run. This record defines the Sample IDs of each sample on a plate, the protocol to apply to each injection and other user-defined items. There are four main methods for preparing a new plate record:

• Manually entering a new plate record

• Manually importing a plate record document

• Automatically importing a plate record document through the barcoding process

• Duplicating information from a completed plate

The following instructions are for manually entering a new plate record. For the other methods, refer to the *Spectrum CE System Operating Manual* #TMD052.

1. Select **New Plate** from the 'Home' screen. This will open a blank 'Plate' screen that is divided into three sections: Plate Information, Sample Information and Injection Information (Figure 4).

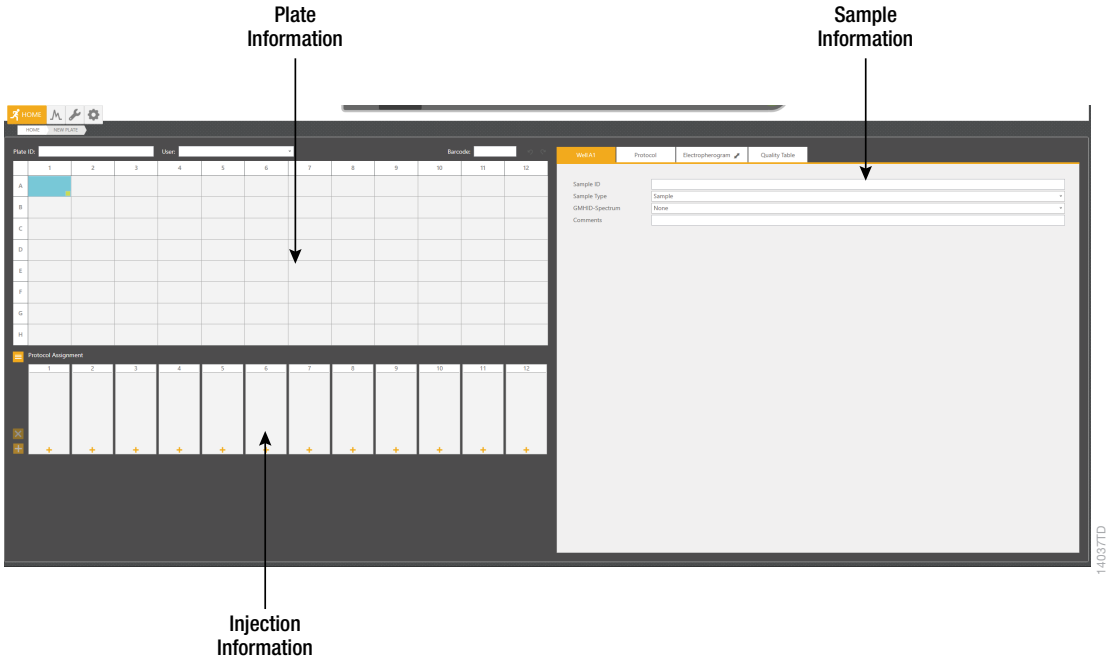


Figure 4. 'Plate' screen.

2. Use the default Plate ID that includes date and time or enter information into the 'Plate ID' field (Figure 5).
3. If desired, enter user information into the 'User' field (Figure 5).

   Note: The 'User' field is a user-defined section that can be used to search or filter previously created or completed plates (refer to the *Spectrum CE System Operating Manual* #TMD052 for information on reviewing plate records). Previously entered user information is available via the drop-down option on the right side of the field (Figure 5).

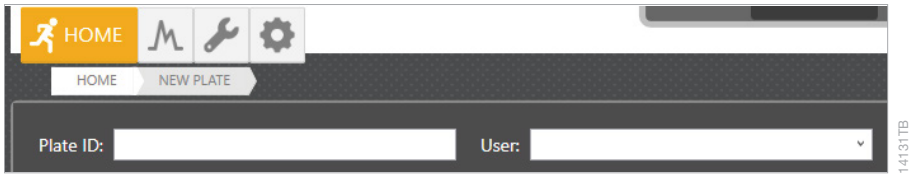


Figure 5. 'Plate ID' and 'User' fields of the plate screen.

4. Enter Sample IDs (sample names) for each sample on the plate.

   Enter a Sample ID directly in the cells of the plate map area of the Plate Information section by selecting the appropriate well(s) and entering the ID information. Right clicking a sample well provides editing options: Cut, Copy, Paste and Clear. There are also Undo and Redo shortcut buttons to the right of the bar code field.

   Alternatively, enter Sample IDs in the 'Sample ID' field of the 'Sample Information' section (Figure 4).

   The green box in the lower right corner of a sample well (Figure 6) can be used to fill other wells with the same information by left clicking and dragging the green box to the other wells.

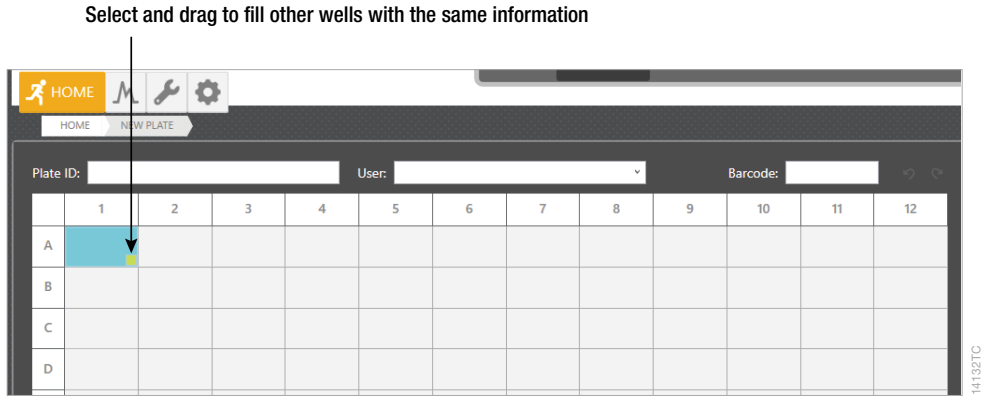


Figure 6. Filling information across multiple sample wells.

5. Select sample types for each sample or group of samples on the plate. Select the sample(s) then use the drop-down menu (Figure 7) to designate the well as Sample, Ladder, Positive Control or Negative Control. These designations will be included in the output file for downstream analysis.

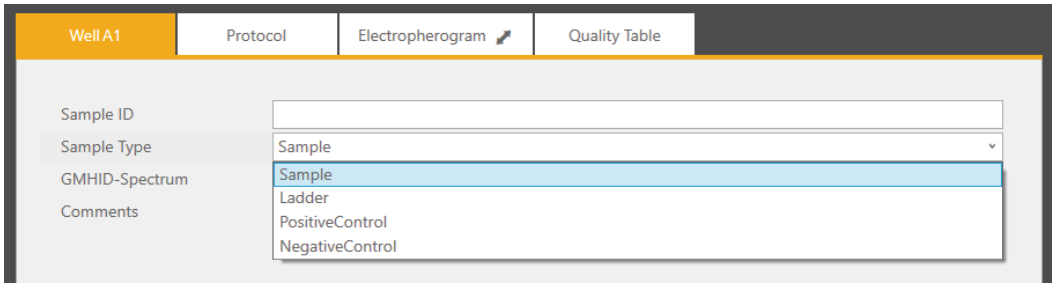


Figure 7. Drop-down options for sample type.

6. **Optional:** Select **PowerPlex_ESX_17_Fast** in the GMHID-Spectrum drop-down menu (Figure 8).

   Notes:

   a. GeneMarker®HID Software for Spectrum CE Systems (GMHID-Spectrum) will generate a Quality Table populated with quality metrics based on the data generated and the template selected. These templates are preloaded and edited within GMHID-Spectrum. Select the appropriate sample type for each well to ensure GMHID-Spectrum functionality. Refer to the *Spectrum CE System Operating Manual* #TMD052 and *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555 for more information.

   b. Selecting the GMHID-Spectrum template does not affect downstream analysis.

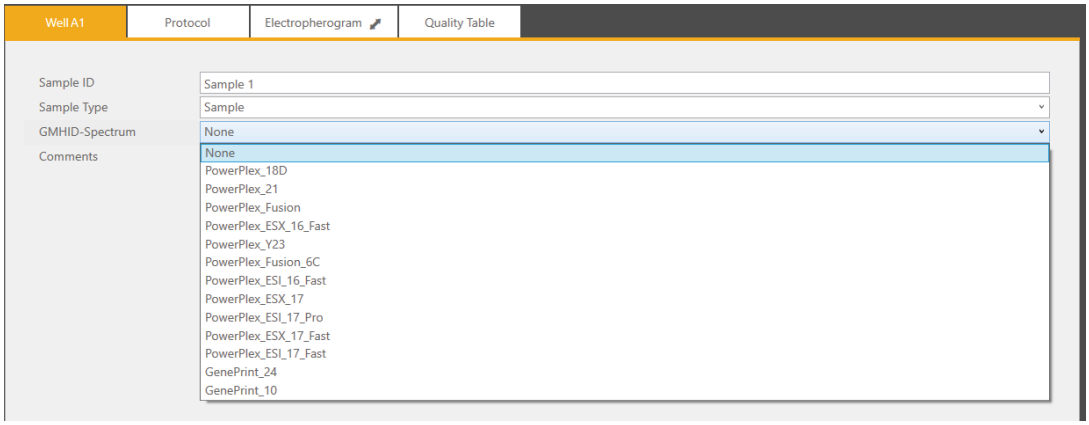


Figure 8. Drop-down options for GMHID-Spectrum run wizard templates.

7. There are three methods to assign run protocol(s) to samples:

   Note: When a run protocol has been assigned to an injection set, an injection number will be assigned and displayed in a circle within the injection set box.

   - To add a run protocol to selected samples, highlight the desired wells (Ctrl + right-click or drag with the mouse). Then right-click, mouse over **Add Injection To Selected Wells** then select the **Promega 5-Dye (5C)** protocol from the pop-up menu.
   - To add a run protocol to individual injections, select the **+** button under the box for that injection set then select the **Promega 5-Dye (5C)** protocol from the pop-up menu.
   - To add a run protocol to multiple injections, highlight the desired injections in the plate map (click-drag, Ctrl + click or click the upper left cell). Then select the **+** button on the left side of the injection information section and choose **Promega 5-Dye (5C)** (Figure 9) from the pop-up menu.

   Notes:

   a. A sample ID must be present in at least one well of an injection set for a run protocol to be assigned to the set.

   b. The Spectrum Control Software includes preloaded run protocols for use with Promega chemistries. Refer to the *Spectrum CE System Operating Manual* #TMD052 for instructions on creating a new protocol or modifying an existing protocol.

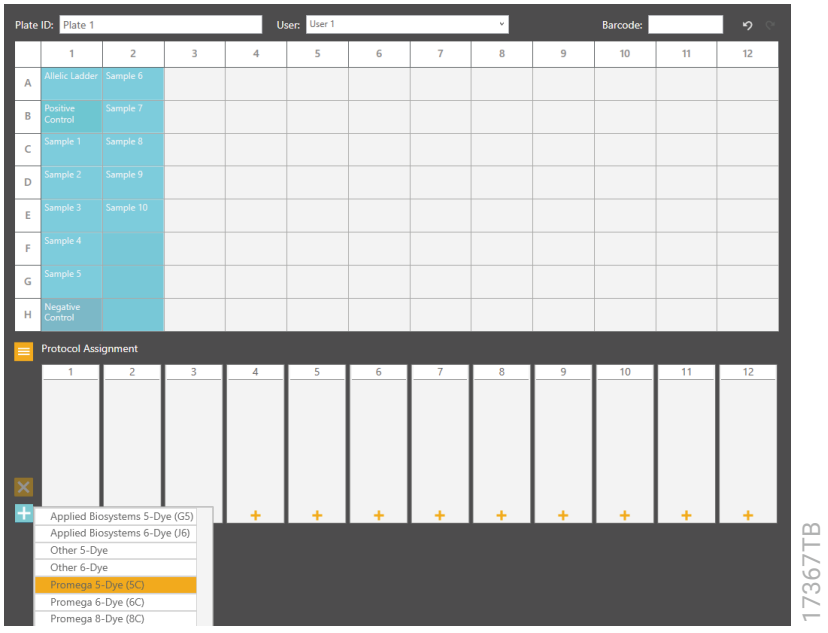


Figure 9. Assigning run protocol to multiple injections.

8. Verify the assigned run protocol(s).

   To verify the parameters of the assigned run protocol(s), select the injection number or a sample well in the plate map then select the 'Protocol' tab of the Sample Information section (Figure 10), which now displays the name of the previously selected protocol. The information in this tab is read-only.

   Note: Refer to the *Spectrum CE System Operating Manual* #TMD052 for instructions on creating or modifying protocols.

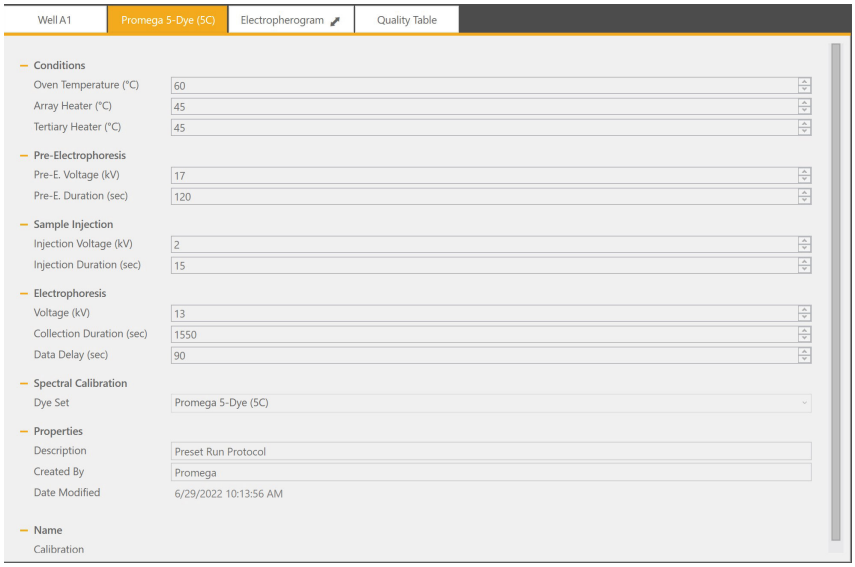


Figure 10. The 'Protocol' tab of the Sample Information section.

9. The plate record is automatically saved and in Draft status until it is linked to a plate position. To access a list of all plates in the Draft status, select the **Draft Plates** button from the 'Home' screen.

5.4 Creating and Loading the Plate Assembly

1. Place the 96-well plate created in Section 5.1 into the Spectrum plate base, lining up the notch above well A12 with the notch on the base.
2. To complete the plate assembly, place the Spectrum plate retainer over the plate/base assembly, lining up the notch on the retainer with the notch on the plate and base. Verify that the retainer is locked in place on both sides of the plate, sitting evenly on top of the base (Figure 11).

Figure 11. The Spectrum plate assembly.

3. Confirm the drawer handle light is illuminated, indicating that the drawer is unlocked and ready for plate loading. Alternatively, check the Message Center to verify that the drawer is unlocked. The Drawer Status indicator is in the Message Center located in the header (Figure 2).
4. Check the Plate Position Status indicator to note which of the four plate positions (A, B, C or D) is open and does not already contain a plate.
5. Open the plate drawer and place the plate assembly in an open plate position, verify that the notch on the plate base retainer aligns with the notch in the plate drawer then close the drawer.

   Notes:

   a. When the plate is seated properly, the Plate Positions indicator will indicate that there is a plate present in the corresponding plate position.

   b. An error message will be displayed if the plate position is occupied by a plate that is being processed.

5.5 Linking a Plate for a Run

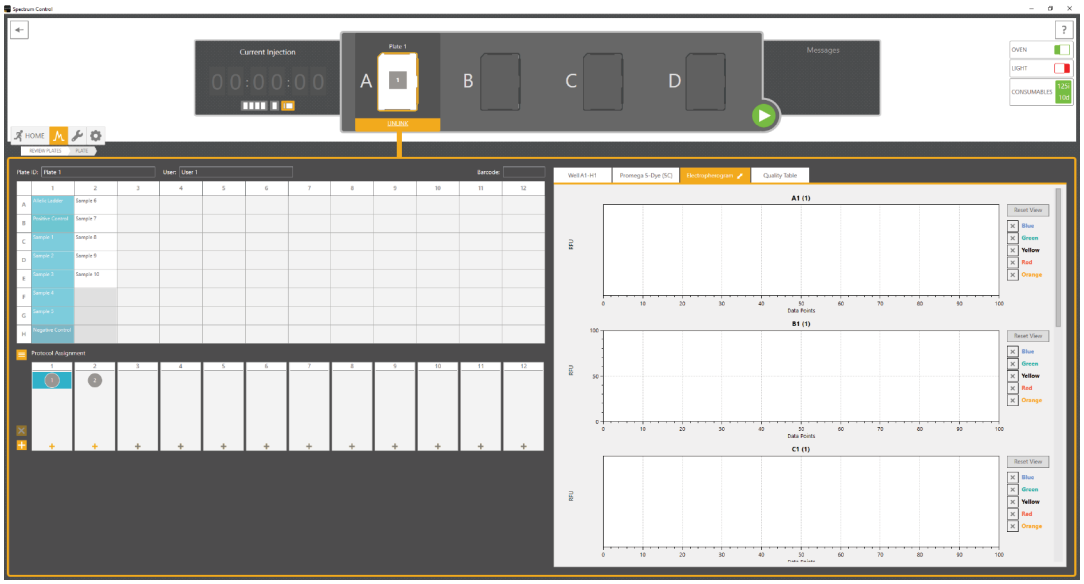
1. A plate record must be linked to the plate before adding it to the run queue.
2. Open the draft plate record, then select **Link** in the Plate Position indicator under the position where the plate was placed. Once the plate is successfully linked, the text under the position will change status to “Unlink” and the Plate ID will appear above the position (Figure 12).
3. Select the **Start** arrow to begin the run.

   Notes:

   a. If a plate is linked to a plate position while the instrument is processing another plate, the newly linked plate will be entered automatically into the run queue.

   b. The status of all linked plates will update in the Plate Positions indicator throughout the run.

   c. To access the plate screen for linked plates, select the plate image in the Plate Positions indicator.



17369TB

Figure 12. Linked plate in the Spectrum Control Software.

5.6 Monitoring a Run

1. The Plate Status is indicated by the icon in the Plate Positions Indicator section of the header.
2. To access the injection status and view data, select the plate image in the header.
   a. The injection status will be displayed in the Injection Information section of the plate record.
   b. To view electropherograms, select a well or column and then select the 'Electropherogram' tab in the Sample Information section.
3. Refer to the *Spectrum CE System Operating Manual* #TMD052 for more information on plate and injection status indicators as well as reordering, adding or removing injections and plates in the run queue.

5.7 Exporting Data

Data from completed injections can be automatically exported as .promega and .fsa files into a defined location on the instrument hard drive. Please confirm that the "Activate Auto Export" check box has been selected in the 'Data Export' tab of the Preferences section. Select **Activate FSA Export** to export .fsa date files in addition to the .promega data files.

Refer to the *Spectrum CE System Operating Manual* #TMD052 for more information on file formats, file location and file naming conventions.

Note: GeneMapper® *ID*-X Software cannot analyze .promega files.

6 Data Analysis Using GeneMapper® *ID*-X Software

GeneMapper® *ID*-X Software Version 1.4 or greater is required for analysis of PowerPlex® ESX 17 Fast data from the Spectrum CE System (.fsa files).

Note: GeneMapper® *ID*-X Software cannot analyze .promega files.

6.1 Importing PowerPlex® ESX 17 Fast Panels, Bins and Stutter Text Files into GeneMapper® *ID*-X Software

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

Getting Started

1. To obtain the proper panels, bins and stutter text files for the PowerPlex® ESX 17 Fast System, go to: **www.promega.com/PowerPlexPanels**
2. Select the PowerPlex® ESX Fast System in the drop-down menu, and then select **GeneMapper ID-X**. Enter your contact information, and then select **Submit**.
3. Save the PowerPlex_ESX_Fast_Panels_IDX_vX.x.txt, PowerPlex_ESX_Fast_Bins_IDX_vX.x.txt and PowerPlex_ESX_Fast_Stutter_IDX_vX.x.txt files, where "X.x" refers to the most recent version of the panels, bins and stutter text files, to a known location on your computer.
4. Save the WEN_ILS_500_IDX.xml file to a known location on your computer.

Importing Panels, Bins and Stutter Files

1. Open the GeneMapper® *ID*-X Software.
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. Select **GeneMapper ID-X Security Group** as the Security Group. This allows access for all users of the software. Other security groups may be used.
7. In the navigation pane, highlight the PowerPlex ESX Fast panels folder that you just imported in Step 5.
8. Select **File**, and then **Import Bin Set**.
9. Navigate to the bins text file downloaded in the Getting Started section above. Select the file, and then **Import**.
10. In the navigation pane, highlight the PowerPlex ESX Fast panels folder that you just imported in Step 5.
11. Select **File**, and then **Import Marker Stutter**. A warning box will appear asking if you want to overwrite current values. Select **Yes**.
12. Navigate to the stutter text file downloaded in the Getting Started section above. Select the file, and then **Import**.
13. At the bottom of the Panel Manager window, select **OK**. This will save the panels, bins and stutter text files, and then close the window.

6.2 Importing the WEN ILS 500 ESS Size Standard into GeneMapper® *ID*-X Software

There are two options when creating a size standard. Use this protocol or the alternative protocol in Section 6.3.

The WEN_ILS_500_IDX.xml file can be used for the WEN ILS 500 ESS size standard supplied with the PowerPlex® ESX 17 Fast System.

1. Select **Tools**, and then **GeneMapper ID-X Manager**.
2. Select the 'Size Standard' tab.
3. Select **Import**.
4. Navigate to the location of the WEN_ILS_500_IDX.xml file downloaded in Section 6.1, Getting Started.
5. Highlight the file, and then select **Import**.
6. Select **GeneMapper ID-X Security Group** as the Security Group. This allows access for all users of the software. Other security groups may be used.
7. Select **Done** to save the changes and close the GeneMapper® *ID*-X Manager.

6.3 Creating a Size Standard with GeneMapper® *ID*-X Software

1. Select **Tools**, and then **GeneMapper ID-X Manager**.
2. Select the 'Size Standard' tab.
3. Select **New**.
4. In the 'Size Standard Editor' window (Figure 13), select **GeneMapper ID-X Security Group** as the Security Group. This allows access for all users of the software. Other security groups may be used.

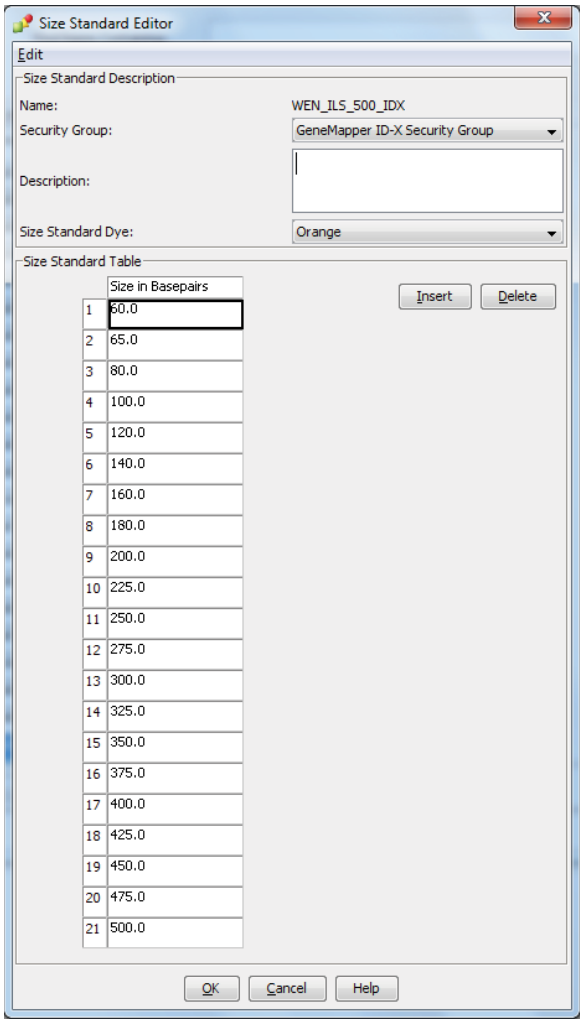


Figure 13. The GeneMapper® *ID*-X Size Standard Editor.

5. Enter a detailed name, such as "WEN_ILS_500_IDX."
6. Choose **Orange** for the Size Standard Dye.
7. Enter the sizes of the internal lane standard fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases). See Section 11.2, Figure 33.
8. Select **OK**.

6.4 Creating a Casework Analysis Method with GeneMapper® *ID*-X Software

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

1. Select **Tools**, and then **GeneMapper ID-X Manager**.
2. Select the 'Analysis Methods' tab.
3. Select **New** and a new analysis method dialog box will open.
4. In the 'Analysis Method Editor' window, select **GeneMapper ID-X Security Group** as the Security Group. This allows access for all users of the software. Other security groups may be used.
5. Enter a descriptive name for the analysis method, such as "PowerPlex ESX 17 Fast."
6. Select the 'Allele' tab (Figure 14).

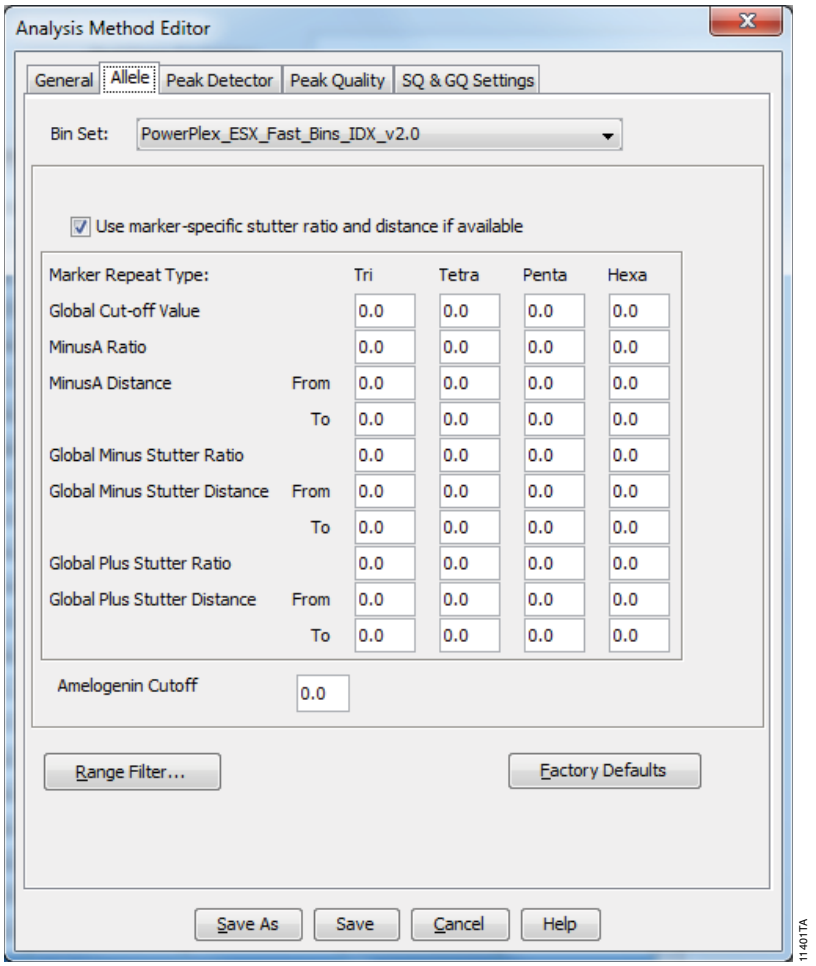


Figure 14. The GeneMapper® *ID*-X 'Allele' tab.

7. Select the bins text file that was imported in Section 6.1.
8. Ensure that the "Use marker-specific stutter ratio and distance if available" box is checked.
9. Select the 'Peak Detector' tab (Figure 15). You will need to optimize these settings. In-house validation should be performed.

   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. Individual laboratories should determine their peak amplitude thresholds from internal validation studies.

   c. The normalization box can be checked or unchecked.

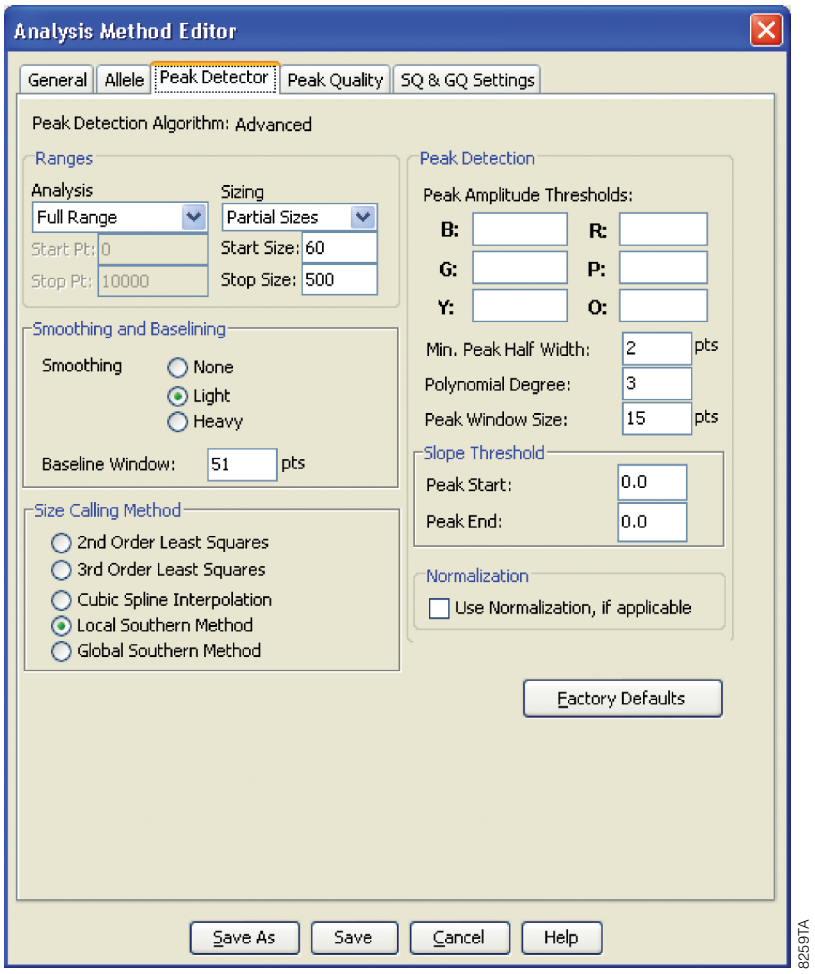


Figure 15. The GeneMapper® *ID*-X 'Peak Detector' tab.

10. Select the 'Peak Quality' tab. You may change the settings for peak quality.
11. Select the 'SQ & GQ Settings' tab. You may change these settings.

    Note: For Steps 10 and 11, see the GeneMapper® *ID*-X user's manual for more information.
12. Select **Save** to save the new analysis method.
13. Select **Done** to exit the GeneMapper® *ID*-X Manager.

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 dESXred files, and then select **Add to List**, followed by **Add**.

   Note: The Spectrum CE System generates .fsa and .promega files for each sample run. The .promega files are not compatible with GeneMapper® *ID*-X Software.
4. The sample types assigned in Section 5.3 will be included in the 'Sample Type' column. If needed, the drop-down menu in the 'Sample Type' column can be used to select **Allelic Ladder**, **Sample**, **Positive Control** or **Negative Control** as appropriate for the sample. Every run folder in the project must contain at least one allelic ladder injection that is designated as **Allelic Ladder** in the 'Sample Type' column for proper genotyping.
5. In the 'Analysis Method' column, select the analysis method created previously in this section.
6. In the 'Panel' column, select the panels text file that was imported in Section 6.1.
7. In the 'Size Standard' column, select the size standard that was imported in Section 6.2 or created in Section 6.3.
8. Select **Analyze** (green arrow button) to start data analysis.

   Note: By default, the software is set to display the 'Analysis Requirements Summary' window if an analysis requirement is not met.
9. If all analysis requirements are met, the 'Save Project' window will open (Figure 16).

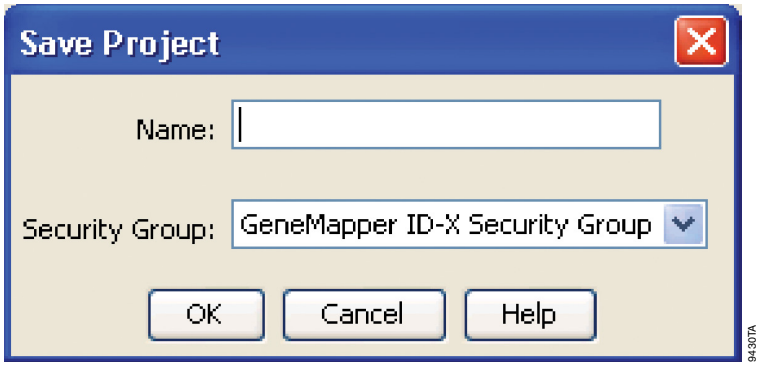


Figure 16. 'Save Project' window.

10. Enter the project name.
11. Choose the applicable security group from the drop-down menu, and then select **OK**.

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

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

6.5 Creating a Databasing or Paternity Analysis Method with GeneMapper® *ID*-X Software

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

1. Select **Tools**, and then **GeneMapper ID-X Manager**.
2. Select the 'Analysis Methods' tab.
3. Select **New**, and a new analysis method dialog box will open.
4. In the 'Analysis Method Editor' window, select **GeneMapper ID-X Security Group** as the Security Group. This allows access for all users of the software. Other security groups may be used.
5. Enter a descriptive name for the analysis method, such as "PowerPlex ESX 17 Fast 20% Filter."

6. Select the 'Allele' tab (Figure 17).
7. Select the bins text file that was imported in Section 6.1.
8. Ensure that the "Use marker-specific stutter ratio and distance if available" box is checked. Doing this will assign locus-specific stutter filters and distances from the imported stutter file. Ensure that the appropriate global filter is applied to this analysis method. For example, for a 20% filter enter "0.20" for the Amelogenin Cuttoff and Global Cutoff Value for Tri and Tetra repeats (Figure 17). You may need to optimize these settings. In-house validation should be performed.

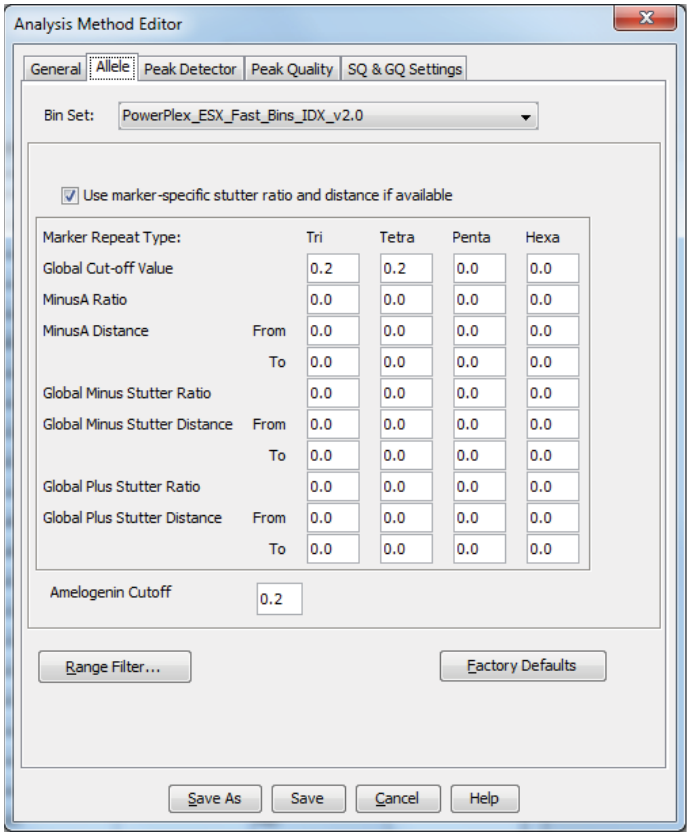


Figure 17. The GeneMapper® *ID*-X 'Allele' tab with settings for using a 20% peak filter.

9. Select the 'Peak Detector' tab (Figure 15). You will need to optimize these settings. In-house validation should be performed.

   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. Individual laboratories should determine their peak amplitude thresholds from internal validation studies.

   c. The normalization box can be checked or unchecked.

10. Select the 'Peak Quality' tab. You may change the settings for peak quality.
11. Select the 'SQ & GQ Settings' tab. You may change these settings.

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

12. Select **Save** to save the new analysis method.
13. Select **Done** to exit the GeneMapper® *ID*-X Manager.

Processing Data for Databasing or Paternity Samples

1. Select **File**, and then **New Project**.
2. Select **Edit**, and then **Add Samples to Project**.
3. Browse to the location of the run files. Highlight the desired files, and then select **Add to List**, followed by **Add**.

   Note: The Spectrum CE System generates .fsa and .promega files for each sample run. The .promega files are not compatible with GeneMapper® *ID*-X Software.

4. The sample types assigned in Section 5.3 will be included in the 'Sample Type' column. If needed, the drop-down menu in the 'Sample Type' column can be used to select **Allelic Ladder**, **Sample**, **Positive Control** or **Negative Control** as appropriate for the sample. Every run folder in the project must contain at least one allelic ladder injection that is designated as **Allelic Ladder** in the 'Sample Type' column for proper genotyping.
5. In the 'Analysis Method' column, select the analysis method created previously in this section.
6. In the 'Panel' column, select the panels text file that was imported in Section 6.1.
7. In the 'Size Standard' column, select the size standard that was imported in Section 6.2 or created in Section 6.3.
8. Select **Analyze** (green arrow button) to start data analysis.

   Note: By default, the software is set to display the 'Analysis Requirements Summary' window if an analysis requirement is not met.

9. If all analysis requirements are met, the 'Save Project' window will open (Figure 16)
10. Enter the project name.
11. Choose the applicable security group from the drop-down menu, and then select **OK**.

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

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

6.6 Controls in GeneMapper® *ID*-X Software

1. Observe the results for the negative control. Using the protocols defined in this manual, the negative control should be devoid of amplification products.
2. Observe the results for the 2800M Control DNA. The expected 2800M DNA allele designations for each locus are listed in Table 6 (Section 11.1).

7 Data Analysis Using GeneMarker®HID Software for Spectrum CE Systems

7.1 Creating an Analysis Method with GeneMarker®HID Software for Spectrum CE Systems

These instructions are intended as a guide to start analyzing data in GeneMarker®HID Software for Spectrum CE Systems (GMHID-Spectrum). They are not intended as comprehensive instructions for using GMHID-Spectrum. More detailed instructions can be found in the *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555.

The current PowerPlex.xml panel files are preloaded in the software or available at **www.promega.com/PowerPlexPanels**.

1. Open GMHID-Spectrum.
2. To access your data files, select **Open Data** in the Magic Wizard or under the File menu.
3. Select **Add**, then navigate to the directory containing your raw data files and select the desired files.

   Note: The Spectrum CE System generates .fsa and .promega files for each sample run. We recommend using the .promega files with GMHID-Spectrum.
4. Select **Open**, and the selected files will appear in the Data File List (Figure 18).

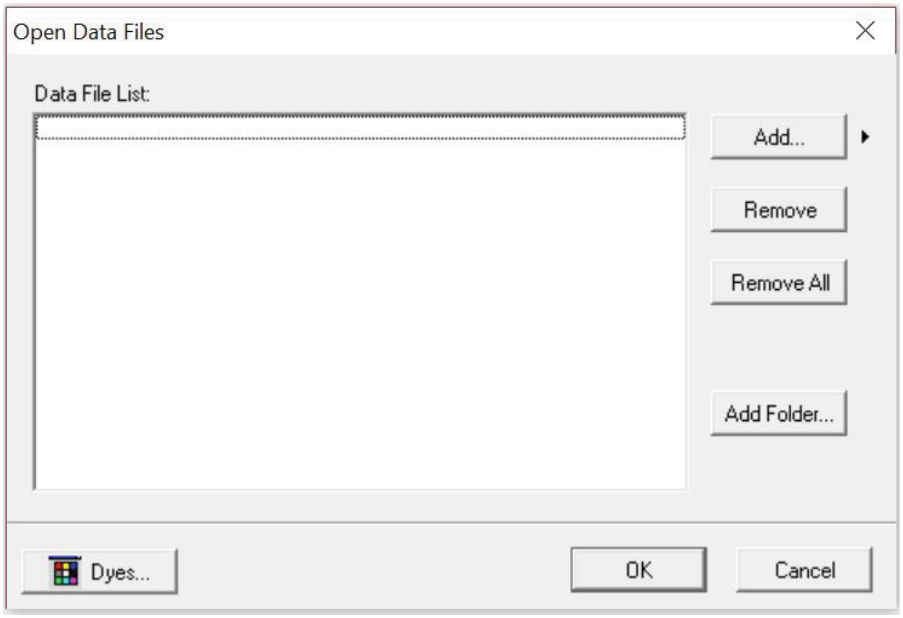


Figure 18. The GMHID-Spectrum Data File List.

5. Select **OK** in the 'Open Data Files' window, and the data will be uploaded into GMHID-Spectrum. In the 'Raw Data' folder in the File Navigator, verify that the sample types (allelic ladder, positive control and negative control) are designated correctly in Section 5. If sample types are not correct, designate sample types by right-clicking on the file name and selecting **Set Sample Type**.
6. Use the Panel Editor in the Tools menu to select the PowerPlex_ESX_17_Fast panel from the Panel Template list. Click on the plus symbol to expand the list, select a marker name, then right-click and select **Edit**. Enter laboratory-specified values for Min Homozygote Intensity, Homozygote Inconclusive Range, Min Heterozygote Intensity, Heterozygote Inconclusive Range, Min Heterozygote Imbalance and Max Heterozygote Imbalance (Figure 19). This will set values for peaks within the marker range. Refer to *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555 for more information.

   Notes:

   a. Individual laboratories should determine their peak amplitude thresholds from internal validation studies.

   b. These settings can be applied to all markers by checking the "'Apply Homo/Hetero Settings to All Markers" check box or by dye channel by checking the "Apply Homo/Hetero Settings to Markers in this dye" check box.
7. Select **OK** and close the 'Panel Editor' window. Select **Save Changes** or **Save as New Panel** under the File menu then close the window.

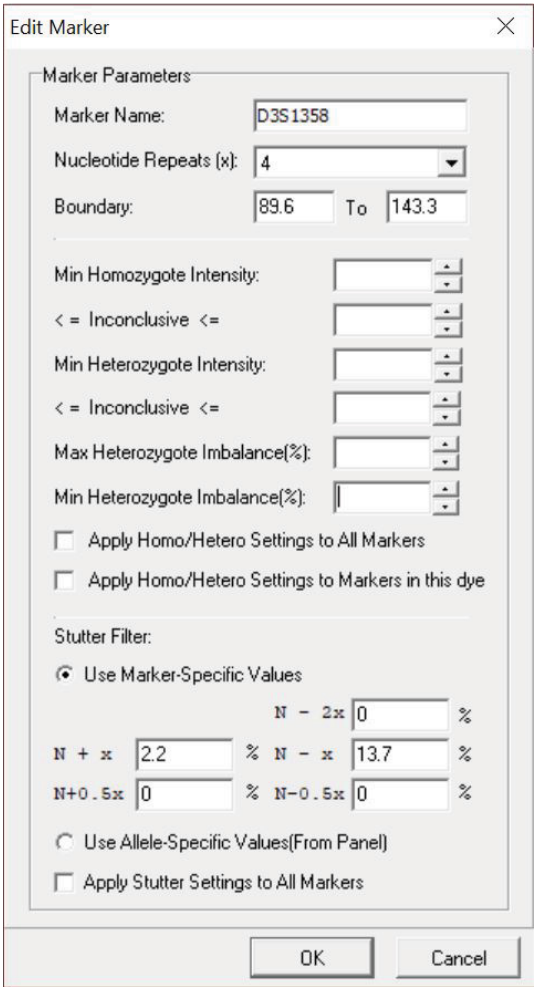


Figure 19. The 'Edit Marker' window for the D3S1358 marker.

8. Select **Run** in the Magic Wizard or choose the **Run Project** icon (green arrow) in the toolbar. The 'Template Selection' screen will appear (Figure 20). Select the PowerPlex_ESX_17_Fast template and the settings shown in Figure 20. Verify the panel that you created in Steps 6 and 7 is selected in the panel drop-down field. The Size Standard must be ILS500 and the Standard Color must be Orange. Select **Next**.

   Note: Changes to the template can be saved at each step during the Run Project process: 'Template Selection', 'Data Process – HID Analysis' and 'Additional Settings – HID Analysis' ' screens.

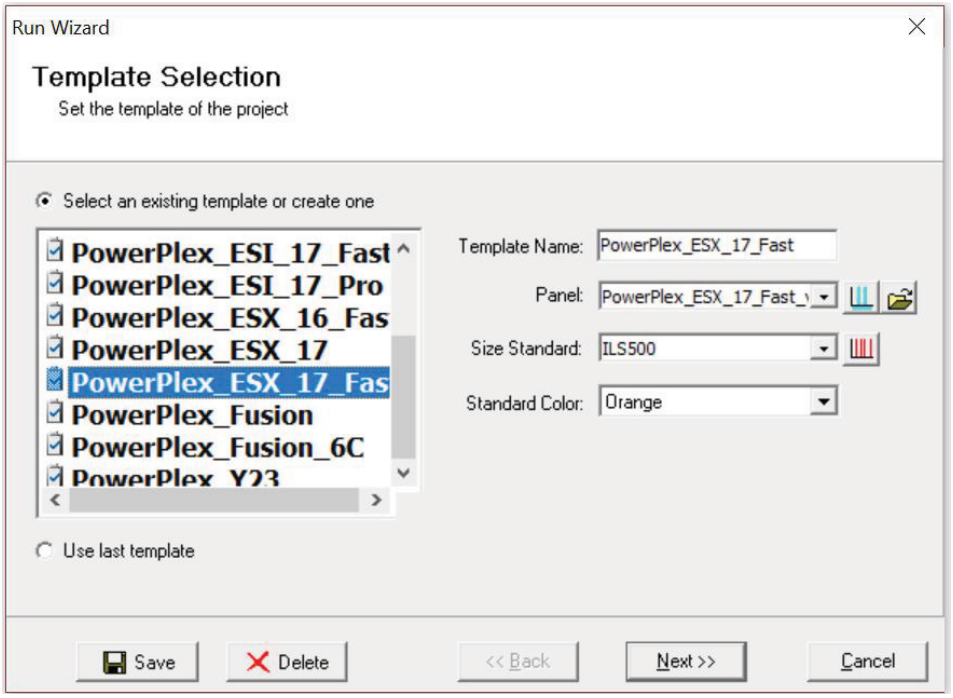


Figure 20. The 'Template Selection' window.

9. The 'Data Process – HID Analysis' window will appear (Figure 21). For the Raw Data Analysis, we recommend using Auto Range, Smooth, Superior Baseline Subtraction and Local Southern for the Size Call.

   For the Allele Call, we recommend using Auto Range. Setting of the Max Intensity (peaks above which will be flagged) and the Min Intensity for Standard Color should be determined by your laboratory. For peaks outside of the panel range, the settings from the panel can be applied by checking the "Apply Nearest Marker Setting" box. Refer to *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555 for more information on use of the Peak Detection Threshold. Select **Next**.

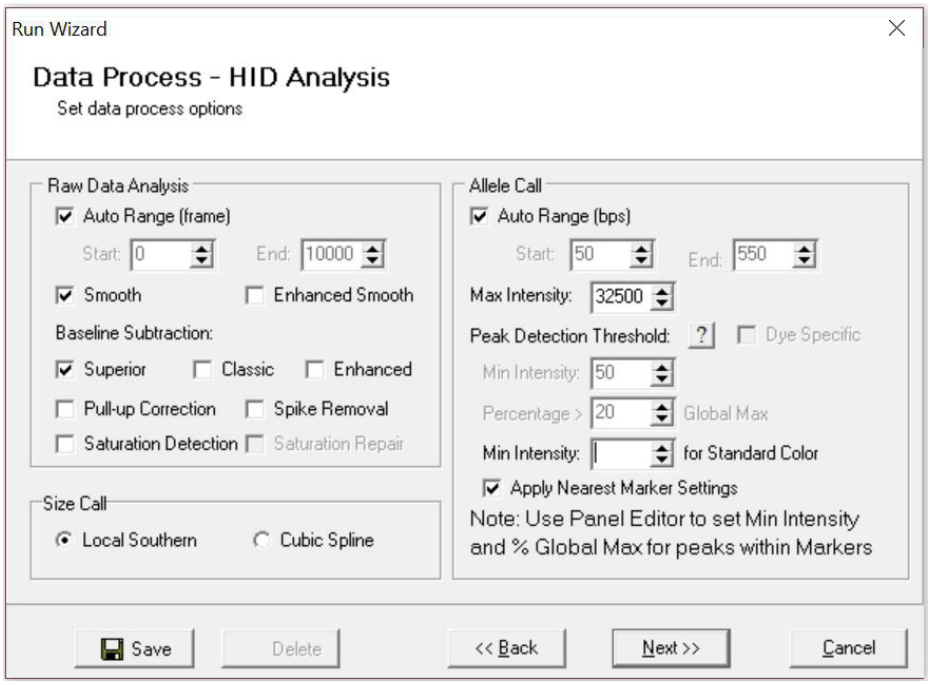


Figure 21. The 'Data Process' window for an analysis method.

10. The 'Additional Settings – HID Analysis' window will appear (Figure 22). Select **ESX_17_2800M** as the P.C. Template 1 and verify that the boxes are checked for "Auto Select Best Ladder" and "Auto Panel Adjustment". The values displayed in the 'Allele Evaluation' dialogue box are defaults and will affect the quality values displayed in the plot settings. For more information on quality values, use of a second positive control and mixture evaluation, refer to the *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555. Select **OK** to analyze the raw data.

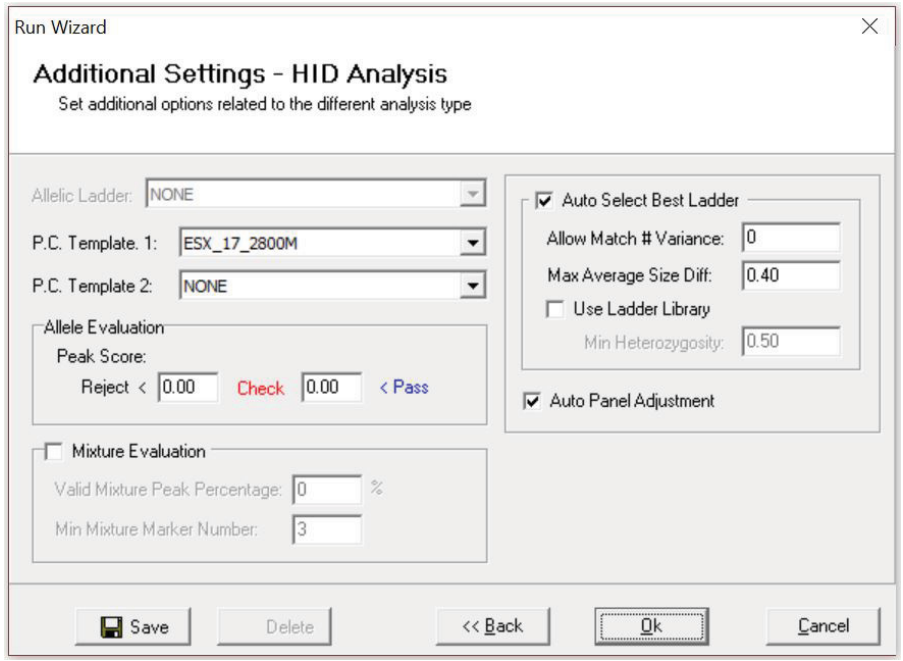


Figure 22. The 'Additional Settings' window.

11. When the analysis is complete, the 'Main Analysis' window will appear. We recommend that you review any yellow or red flagged markers and address them according to your laboratory's standard operating procedures.

    Note: Quality and display settings are set using Preferences under the View menu. Refer to *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555 for more information.

7.2 Controls in GeneMarker®HID Software for Spectrum CE Systems

1. Observe the results for the negative control. Using the protocols defined in this manual, the negative controls should be devoid of amplification products.
2. Observe the results for the 2800M Control DNA. The expected 2800M allele designations for each locus are listed in Table 6 (Section 11.1).

8 Results

Representative results of the PowerPlex® ESX 17 Fast System are shown in Figure 23. The PowerPlex® ESX 17 Fast Allelic Ladder Mix is shown in Figure 24.

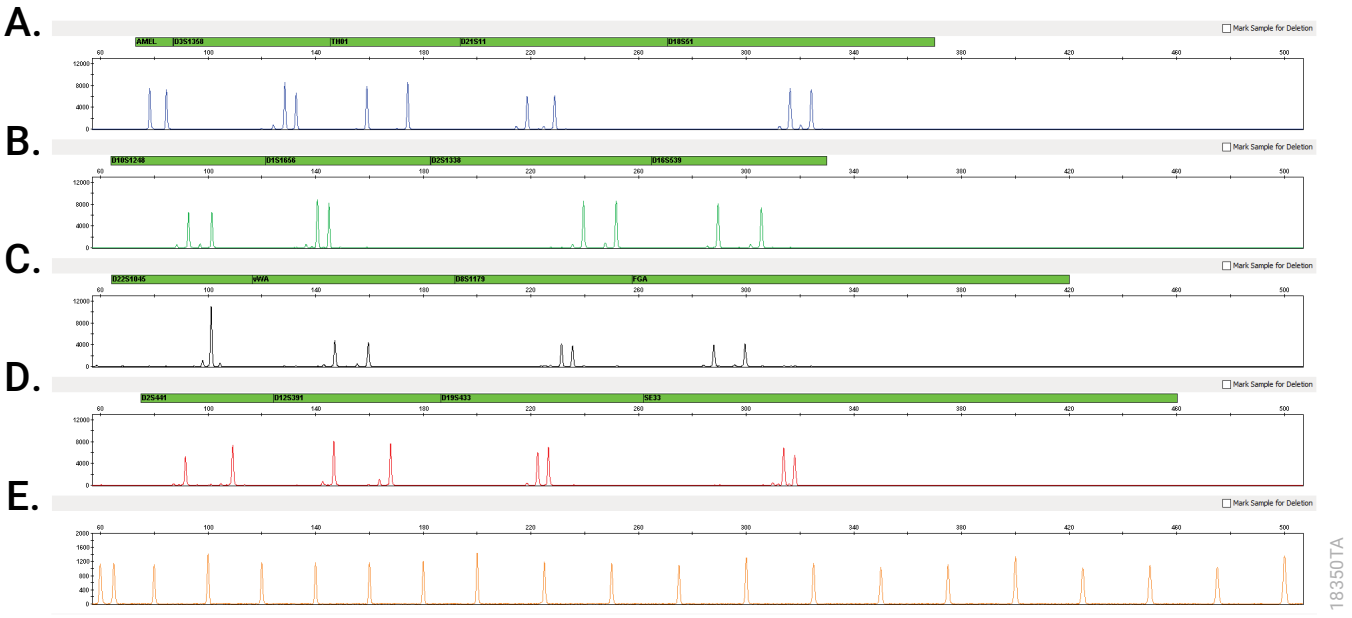


Figure 23. The PowerPlex® ESX 17 Fast System. The 2800M Control DNA (0.5ng) was amplified using the PowerPlex® ESX 17 Fast System. Amplification products were mixed with WEN Internal Lane Standard 500 ESS and analyzed with a Spectrum CE System and a 2kV, 15-second injection. Results were analyzed using GeneMapper® *ID*-X software, version 1.4, and PowerPlex® ESX Fast panels and bins text files. **Panel A.** An electropherogram showing the peaks of the fluorescein-labeled loci: Amelogenin, D3S1358, TH01, D21S11 and D18S51. **Panel B.** An electropherogram showing the peaks of the JOE-labeled loci: D10S1248, D1S1656, D2S1338 and D16S539. **Panel C.** An electropherogram showing the peaks of the TMR-ET-labeled loc: D22S1045, vWA, D8S1179 and FGA. **Panel D.** An electropherogram showing the peaks of the CXR-ET-labeled loci: D2S441, D12S391, D19S433 and SE33. **Panel E.** An electropherogram showing the 60–500bp fragments of the WEN Internal Lane Standard 500 ESS.

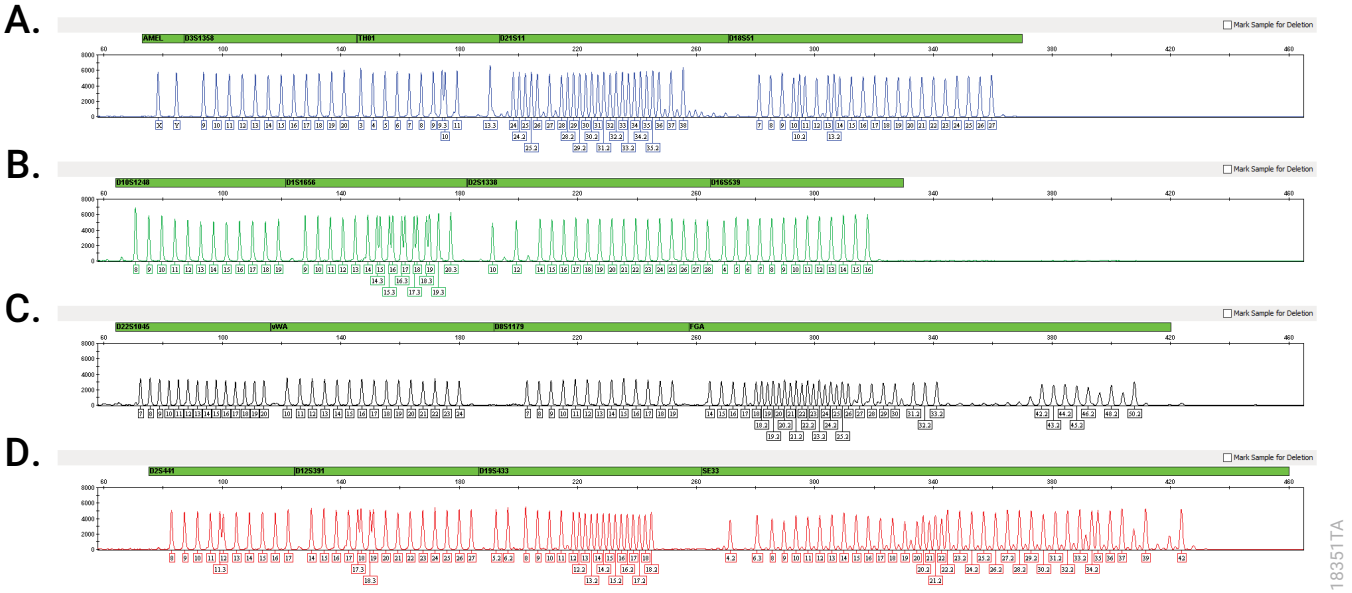


Figure 24. The PowerPlex® ESX 17 Fast Allelic Ladder Mix. The PowerPlex® ESX 17 Fast Allelic Ladder Mix was analyzed with a Spectrum CE System and a 2kV, 15-second injection. The sample file was analyzed with the GeneMapper® *ID*-X software, version 1.4, and PowerPlex® ESX Fast panels and bins text files. **Panel A**. The fluorescein-labeled allelic ladder components and their allele designations. **Panel B.** The JOE-labeled allelic ladder components and their allele designations. **Panel C.** The TMR-ET- labeled allelic ladder components and their allele designations. **Panel D.** The CXR-ET-labeled allelic ladder components and their allele designations.

Artifacts and Stutter

Stutter products are a common amplification artifact associated with STR analysis (15,16). Stutter products are often observed one repeat unit below the true allele peak and, occasionally, two repeat units smaller or one repeat unit larger than the true allele peak. Frequently, alleles with a greater number of repeat units will exhibit a higher percent stutter. The pattern and intensity of stutter may differ slightly between primer sets for the same loci.

Increased stutter often is associated with D22S1045, as it is a trinucleotide repeat marker.

The mean stutter plus three standard deviations observed at each locus is used in the PowerPlex® ESX Fast stutter text file for locus-specific filtering in GeneMapper® ID-X software and in the panel file included in GMHID-Spectrum.

In addition to stutter peaks, you may observe the following low-level DNA -dependent artifact peaks (Table 4) and DNA-independent (with or without human genomic DNA) artifact peaks (Table 5) with the PowerPlex® ESX 17 Fast System loci.

Table 4. DNA-Dependent Artifacts Observed in Amplification Reactions with Human Genomic DNA.

Locus	Artifact Size[1]
Amelogenin	n–1, n+1
D21S11	n–2, n+2
D1S1656	n–2, n+2
D2S441	n–2, n+2
SE33	n–2, n+2

Table 5. DNA-Independent Artifacts Observed in Amplification Reactions with and without Human Genomic DNA.

Dye Label	Artifact Size[1]
Fluorescein	~63–67 bases
TMR-ET	~57–62 bases
	D22S1045 at allele 6

[1]Artifact sizes may vary depending on CE instrumentation and environmental conditions in the laboratory.

9 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**

Refer to the *Spectrum CE System Operating Manual* #TMD052 for instrument troubleshooting. For troubleshooting GMHID-Spectrum, refer to the *GeneMarker®HID Software for Spectrum CE Systems User Manual* #TM555.

9.1 Amplification and Fragment Detection

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 protocol. 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.
Faint or absent allele 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. 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 source 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 or a freezer plate block or in an ice-water bath immediately prior to capillary electrophoresis. Do not cool samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA reannealing. 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 reannealing.
	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.
	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.
	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.

Symptoms	Causes and Comments
Allelic ladder not running the same as samples	Be sure the allelic ladder and samples are from the same instrument run.
	Allelic ladder and primer pair mix were not compatible. Ensure that the allelic ladder is from the same kit as the primer pair mix.
	Migration of samples changed slightly over the course of a CE run with many samples. This may be due to changes in temperature 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. Include one allelic ladder for every 16 samples.
	Internal size standard was not assigned correctly. Evaluate the sizing labels on the ILS and correct if necesary.
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.

9.2 Amplification of Extracted DNA

The following information is specific to amplification of purified DNA. For information about general amplification and detection, see Section 9.1.

Symptoms	Causes and Comments
Faint or absent allele peaks	Impure template DNA. Depending on the DNA purification procedure used and sample source, inhibitors might be present in the DNA sample. This may be more of an issue as DNA sample volume increases as a percentage of the total amplification reaction volume.
	Insufficient template. Use the recommended amount of template DNA if available. Quantify template DNA before use if possible.
	High salt concentration or altered pH. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the DNA volume should not exceed 20% of the total reaction volume. Carryover of K^+, Na^+, Mg^{2+} or EDTA from the DNA sample can negatively affect PCR. A change in pH also may affect PCR. Store DNA in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or TE^{-4} buffer with 20µg/ml glycogen.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl for extracted DNA. Decreasing the reaction volume may result in suboptimal performance.

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels	Amplification of more than the recommended amount of purified DNA can result in a higher number of artifact peaks due to overamplification, resulting in saturating signal. Use the recommended amount of template DNA. See Results section for additional information about stutter and artifacts. The amount of template will need to be optimized if you are using reduced reaction volumes.
	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 the amount of template DNA. Using more than the recommended amount of template DNA can result in incomplete adenylation. • Decrease cycle number. • Increase the final extension time.
Peak height imbalance	Amplification of greater than the recommended amount of template can result in an imbalance, with smaller loci showing more product than larger loci. Use less template or fewer cycles. The amount of template will need to be optimized if you are using reduced reaction volumes.
	Degraded DNA sample. DNA template was degraded, and larger loci showed diminished yield.
	Insufficient template DNA. Use the recommended amount of template DNA if available. Stochastic effects can occur when amplifying low amounts of template.
	Impure template DNA. Inhibitors that may be present in forensic samples can lead to allele dropout or imbalance.
	Imbalance may be seen more often when using the maximum template volume or a reduced amplification reaction volume.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl. Decreasing the reaction volume may result in suboptimal performance.

9.3 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 9.1.

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 released 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 storage card. Take punches from a different portion of the card. Increasing cycle number can increase 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.
	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 amplification reactions with nonlytic 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 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 reagent activity.

Symptoms	Causes and Comments
Faint or absent allele peaks in 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 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.
Extra peaks visible in one or all color channels	Punch 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.

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 recommendations 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.
	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 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 amplification reactions 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 12.5µl or 25µl amplification reaction. Reducing the PunchSolution™ Reagent volume may improve results for reactions with reduced amplification volumes. Optimization and validation are required.
	Inactive PunchSolution™ Reagent was used to pretreat nonlytic 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.

Symptoms	Causes and Comments
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 punches but does not normalize the amount of DNA present.

9.4 Direct Amplification of DNA from Swabs

The following information is specific to direct amplification of DNA from swabs after pretreatment using the SwabSolution™ Kit. For additional information about general amplification and detection, see Section 9.1.

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 reagent 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.
	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 released from cellular proteins.

Symptoms	Causes and Comments
Faint or absent peaks for the positive control reaction	If the positive control reaction failed to amplify, check to make sure that the correct amount of 2800M Control DNA was added to the reaction. Due to the reduced cycle numbers used with swab extracts, it is necessary to increase the mass of 2800M Control DNA to obtain a profile. Use the recommended amount of 2800M Control DNA per amplification reaction. This mass of DNA should be reduced if 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.
	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.

Symptoms	Causes and Comments
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 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.
	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 re-freeze, 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 released 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, quantitate the DNA using a fluorescence-based double-stranded DNA quantitation method or qPCR-based quantitation method. The quantitation values can be used to normalize input template amounts to minimize variation in signal intensity.

9.5 GeneMapper® *ID*-X Software

Symptoms	Causes and Comments
Stutter peaks not filtered	Stutter text file was not imported into the Panel Manager when the panels and bins text files were imported.
	Be sure that the "Use marker-specific stutter ratio and distance if available" box is checked. If the "Use marker-specific stutter ratio and distance if available" box is not checked, stutter distance must be defined in the Analysis Method 'Allele' tab.
Samples in the project not analyzed	The 'Analysis Requirement Summary' window was not active, and there was an analysis requirement that was not met. Turn on 'Analysis Requirement Summary' in the 'Project Options' menu under **File**, and correct the necessary analysis requirements to continue analysis.
Edits in label edit viewer cannot be viewed	To view edits made to a project, the project first must be saved. Close the plot view window, return to the main GeneMapper® *ID*-X page and save the project. Display the plot window again, and then view the label edit table.
Marker header bar for some loci are gray	When an edit is made to a locus, the quality flags and marker header bar automatically changes to gray. To change the GQ and marker header bar for a locus to green, override the GQ in the plot window.
Alleles not called	To analyze samples with GeneMapper® *ID*-X software, at least one allelic ladder must be defined per folder of sample files being analyzed in the project.
	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. Re-analyze samples with an allelic ladder from the same run.
	The GeneMapper® *ID*-X software requires that the allelic ladder be imported from the same folder as the sample. Be sure that the allelic ladder is in the same folder as the sample. Create a new project and re-analyze as described in the section 'Processing Data for Casework Samples' or 'Processing Data for Databasing or Paternity Samples.'
	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 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.
	Incorrect polymer was used. Use of a polymer other than Polymer4 may change migration of the fragments, causing alleles to migrate outside of the panel range.
Size standard not called correctly	Starting data point was incorrect for the partial range chosen for the analysis in the GeneMapper® *ID*-X Peak Detector tab as described in sections 'Creating a Casework Analysis Method' or 'Creating a Databasing or Paternity Analysis Method.' Adjust the starting data point or use a full range for the analysis.
	Extra peaks in size standard. Open the Size Match Editor. Highlight the extra peak, select **Edit** and select **Delete Size Label**. Select **Auto-Adjust Sizes**.
	An incorrect size standard was used.
	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.

Symptoms	Causes and Comments
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.
Significantly raised baseline	Poor spectral calibration. Perform a new spectral calibration and rerun the samples.
	Confirm that the correct spectral was used to run the samples.

9.6 GeneMarker® HID Software for Spectrum CE Systems

Symptoms	Causes and Comments
Stutter peaks not filtered	Be sure the "Use Marker-Specific Values" box in the Panel Editor is checked. Alternatively, select the "Use Allele-Specific Values (From Panel)" box in the 'Edit Marker' window and enter the appropriate stutter filters in the Panel Table of the 'Panel Editor' window.
Alleles not called	To analyze samples with GMHID-Spectrum, at least one allelic ladder must be present in the data set. Ensure that the allelic ladder is designated as such in Section 6 or 7.
	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. See the 'Off-ladder or off-bin alleles' column below for more information.

Symptoms	Causes and Comments
Off-ladder or off-bin alleles	An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.
	The panel file selected for analysis was incorrect for the STR system used. Assign a correct panel file that corresponds to the STR system used for amplification.
	The allelic ladder was not designated as an allelic ladder in Section 6 or 7.
	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. Allelic ladders are flagged for Analysis Quality when: • There is an error in bin shifting • Peaks in virtual bins have greater than expected peak heights • The expected peaks cannot be identified in a marker. When expected peaks cannot be identified in a marker, the marker bar will be shaded dark green, indicating the marker failed analysis. When errors in bin shifting occur or when peaks in virtual bins have higher than expected peak heights, the marker bars will be outlined in red. Any peaks that cause an 'Analysis Quality' flag are indicated in the electropherogram and Peak Table. When Flag Variant Alleles in Ladder is selected in the 'Display' tab of the 'Preferences' window, peaks in virtual bins with higher-than-expected peak heights are indicated by yellow vertical bars and green allele labels. You can set your preferences to automatically delete these peaks by selecting Auto-Delete Alleles in Virtual Bins in Allelic Ladder in the 'Forensics' tab of the 'Preferences' window. Peaks that are not present in the expected bins are marked with red vertical bars and red allele labels

Symptoms	Causes and Comments
Size standard not called or poor quality	If a partial range was chosen for the Allele Call analysis in the “Data Process - HID Analysis’ window of the Run Wizard, the chosen starting or end point was incorrect. Adjust the starting point or end point or use the Auto Range setting for the analysis. The incorrect Standard Color was selected in the ‘Template Selection’ window of the Run Wizard. The Standard Color should be Brown.
	If the quality of the size standard peaks is not sufficient (and the ‘Low ILS Quality Flag SQ’ section in the ‘Sample Quality’ tab of the ‘Preferences’ window is selected), the size standard is marked with a red SQ flag in the Sample File Tree of the ‘Main Analysis’ window and in the upper left corner of the sample electropherogram. There are three criteria that determine whether a size standard fails the size quality requirements: • The sample size standard does not contain all peaks that are marked as “Enabled” in the chosen size standard template. • Not all of the expected size standard peaks are called in the ‘Main Analysis’ window. If the expected size standard peaks are present but are not labeled, open the Calibration Charts and select the affected sample from the Sample List. Edit the size calls of the sample size standard to remove the extra peak(s) and define the correct peak(s). Right-click anywhere in the Sample Size Standard Chart and select **Update Calibration**. • Not all of the expected size standard peaks are of high quality such that the absolute difference in size between the actual size standard peaks and the expected, Enabled size standard peaks is less than 0.2bp.
	An incorrect size standard was used.
	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.

Symptoms	Causes and Comments
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.
Significantly raised baseline	Poor spectral calibration. Perform a new spectral calibration and rerun the samples.
	Confirm that the correct spectral was used to run the samples.

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11 Appendix

11.1 Advantages of Using the Loci in the PowerPlex® ESX 17 Fast System

The loci included in the PowerPlex® ESX 17 Fast System (Table 6 and Table 7) were selected because they meet the recommendations of the European Network of Forensic Science Institutes (ENFSI). The PowerPlex® ESX 17 Fast System amplifies all ENFSI core loci plus SE33 in a single reaction. Table 8 lists the PowerPlex® ESX 17 Fast System alleles amplified from the 2800M Control DNA.

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

Terminal nucleotide addition (17,18) occurs when a thermostable nonproofreading DNA polymerase adds a nucleotide, generally adenine, to the 3´ ends of amplified DNA fragments in a template-independent manner. The efficiency with which this occurs varies with different primer sequences. Thus, an artifact peak one base shorter than expected (i.e., missing the terminal addition) is sometimes seen. We have modified primer sequences and added a final extension step of 60°C (19) to the amplification protocols to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of template DNA are used.

Table 6. The PowerPlex® ESX 17 Fast System Locus-Specific Information.

STR Locus	Label	Chromosomal Location[1]	Repeat Sequence[2] 5´→3´
D18S51	Fluorescein	18q21.33 (59.1Mb)	AGAA (20)
D21S11	Fluorescein	21q21.1 (19.476Mb)	TCTA Complex (20)
TH01	Fluorescein	11p15.5 (2.149Mb))	AATG (20)
D3S1358	Fluorescein	3p21.31 (45.557Mb)	TCTA Complex
Amelogenin[3]	Fluorescein	Xp22.1–22.3 and Y	NA
D16S539	JOE	16q24.1 (84.944Mb)	GATA
D2S1338	JOE	2q35 (218.705Mb)	TGCC/TTCC
D1S1656	JOE	1q42 (228.972Mb)	TAGA Complex
D10S1248	JOE	10q26.3 (130.567Mb)	GGAA
FGA	TMR-ET	4q28 (155.866Mb)	TTTC Complex (20)
D8S1179	TMR-ET	8q24.13 (125.976Mb)	TCTA Complex (20)
vWA	TMR-ET	12p13.31 (5.963Mb)	TCTA Complex (20)
D22S1045	TMR-ET	22q12.3 (35.779Mb)	ATT
SE33	CXR-ET	6q14 (89.043Mb)	AAAG Complex
D19S433	CXR-ET	19q12 (35.109Mb)	AAGG Complex
D12S391	CXR-ET	12p12 (12.341Mb)	AGAT/AGAC Complex
D2S441	CXR-ET	2p14 (68.214Mb)	TCTA

[1]Information about chromosomal location of these loci can be found in references 21 and 22, and at: **www.cstl.nist.gov/biotech/strbase/chrom.htm**

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

[3]Amelogenin is not an STR.

NA = Not Applicable

Table 7. The PowerPlex® ESX 17 Fast System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components[1,2] (bases)	Repeat Numbers of Allelic Ladder Components[3]
D18S51	Fluorescein	286–366	7–10, 10.2, 11–13, 13.2, 14–27
D21S11	Fluorescein	203–259	24, 24.2, 25, 25.2, 26–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38
TH01	Fluorescein	152–195	3–9, 9.3, 10–11, 13.3
D3S1358	Fluorescein	103–147	9–20
Amelogenin[4]	Fluorescein	89, 95	X, Y
D16S539	JOE	273–321	4–16
D2S1338	JOE	197–269	10, 12, 14–28
D1S1656	JOE	137–184	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3
D10S1248	JOE	83–127	8–19
FGA	TMR-ET	264–410	14–18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 48.2, 50.2
D8S1179	TMR-ET	203–251	7–19
vWA	TMR-ET	124–180	10–24
D22S1045	TMR-ET	79–118	7–20
SE33	CXR-ET	267–417	4.2, 6.3, 8–20, 20.2, 21, 21.2, 22, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35–37, 39, 42
D19S433	CXR-ET	193–245	5.2, 6.2, 8–12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18, 18.2
D12S391	CXR-ET	130–182	14–17, 17.3, 18, 18.3, 19–27
D2S441	CXR-ET	88–124	8–11, 11.3, 12–17

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

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

[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 8. The PowerPlex® ESX 17 Fast System Allele Determinations for the 2800M Control DNA.

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

11.2 The WEN Internal Lane Standard 500 ESS

The WEN Internal Lane Standard 500 ESS contains 21 DNA fragments of 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases in length (Figure 25). Each fragment is labeled with WEN dye and can be detected separately (as a fifth color) in the presence of PowerPlex® ESX 17 Fast-amplified material. The WEN ILS 500 ESS is designed for use in each CE injection to increase precision in analyses when using the PowerPlex® ESX 17 Fast System. Protocols to prepare and use this internal lane standard are provided in Section 5.

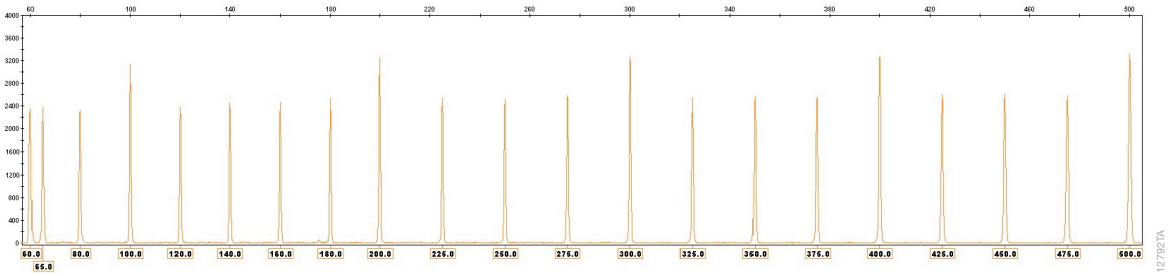

Figure 25. WEN Internal Lane Standard 500 ESS. An electropherogram showing the WEN Internal Lane Standard 500 ESS fragments.

11.3 Direct Amplification of DNA from Storage Card Punches in a 12.5µl Reaction Volume

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

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 with a gold-plated silver or silver sample block 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
- PunchSolution™ Kit (Cat.# DC9271) for nonlytic card punches
- 5X AmpSolution™ Reagent (Cat.# DM1231, also supplied with the PunchSolution™ Kit)
- 1.2mm Harris Micro-Punch or equivalent manual punch and cutting mat or automated punch system

Notes:

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

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

Lytic card sample types include:

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

Nonlytic card sample types include:

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

Pretreat nonlytic sample types with the PunchSolution™ Kit (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 card punches, adding PCR amplification mix to the well before adding the punch may help alleviate static problems. For nonlytic card punches, adding PunchSolution™ Reagent to the well before adding the punch during pretreatment may help alleviate static problems.

Amplification Setup

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

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

2. Vortex the 5X AmpSolution™ Reagent for 10–15 seconds.

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

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

4. Use a clean plate for reaction assembly, and label it appropriately. Alternatively, determine the number of clean 0.2ml reaction tubes required and label them appropriately.
5. Add the final volume of each reagent listed in Table 9 to a clean tube.

Table 9. PCR Amplification Mix for Direct Amplification of DNA from Storage Card Punches in a 12.5µl Reaction Volume

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	6.25µl	×		=	
PowerPlex® ESI/ESX Fast 5X Master Mix	2.5µl	×		=	
PowerPlex® ESX 17 Fast 10X Primer Pair Mix	1.25µl	×		=	
5X AmpSolution™ Reagent	2.5µl	×		=	
total reaction volume	12.5µl				

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

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

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

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

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

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

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

   Notes:

   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 depending on thermal cycling conditions and laboratory preferences. Typically, 5ng of 2800M Control DNA is sufficient to provide a robust profile using the cycle numbers recommended here. A one-cycle reduction in cycle number will require a twofold increase in mass of DNA template to generate similar signal intensity. Similarly, a one-cycle increase in cycle number will require a twofold reduction in the amount of 2800M Control DNA to avoid signal saturation.

9. Reserve a well containing PCR amplification mix as a negative amplification control.

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

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

Thermal Cycling

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

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

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

   Notes:

   a. When using the ProFlex® PCR System, use the 9700 Simulation Mode as the ramp speed.

   b. When using the GeneAmp® PCR System, the program must be run with Max Mode as the ramp speed. This requires a gold-plated silver or silver sample block. The ramp speed is set after the thermal cycling run is started. When the 'Select Method Options' screen appears, select **Max** for the ramp speed and enter the reaction volume.

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

3. After completion of the thermal cycling protocol, proceed with 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 and instrumentation.

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.
2. Depending on your preferred protocol, place one 1.2mm storage card punch containing buccal cells or one 1.2mm punch of a storage card containing whole blood into each well of a reaction plate. Be sure to 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 (24, 25 and 26 cycles).
5. Following amplification, use your laboratory's validated separation and detection protocols to determine the optimal cycle number for the sample type.

11.4 Direct Amplification of DNA from Swabs in a 12.5µl Reaction Volume

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

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 with a gold-plated silver or silver sample block 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
- SwabSolution™ Kit (Cat.# DC8271)

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

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

Amplification Setup

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

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

2. Vortex the 5X AmpSolution™ Reagent for 10–15 seconds.

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

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

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

5. Add the final volume of each reagent listed in Table 10 to a clean tube.

Table 10. PCR Amplification Mix for Direct Amplification of DNA from Swabs in a 12.5µl Reaction Volume.

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	4.25µl	×		=	
PowerPlex® ESI/ESX Fast 5X Master Mix	2.50µl	×		=	
PowerPlex® ESX 17 Fast 10X Primer pair Mix	1.25µl	×		=	
5X AmpSolution™ Reagent	2.50µl	×		=	
swab extract	2.00µl				
total reaction volume	12.50µl				

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

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

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

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

7. Pipet 2µl of swab extract for each sample into the appropriate well of the reaction plate.
8. For the positive amplification control, vortex the tube of 2800M Control DNA, and then dilute an aliquot to 2.5ng/µl. Add 2µl (5ng) to a reaction well containing 10.5µl of PCR amplification mix.

   Note: Optimization of the amount of 2800M Control DNA may be required depending on thermal cycling conditions and laboratory preferences.
9. For the negative amplification control, pipet 2µl of 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.
10. Seal or cap the plate, or close the tubes.
    Optional: Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

Thermal Cycling

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

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

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

   Notes:

   a. When using the ProFlex® PCR System, use the 9700 Simulation Mode as the ramp speed.

   b. When using the GeneAmp® PCR System 9700, the program must be run with Max Mode as the ramp speed. This requires a gold-plated silver or silver sample block. The ramp speed is set after the thermal cycling run is started. When the 'Select Method Options' screen appears, select **Max** for the ramp speed and enter the reaction volume.

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

3. After completion of the thermal cycling protocol, proceed with 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 and instrumentation.

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.
2. Prepare three identical reaction plates with aliquots of the same swab extracts.
3. Amplify samples using the thermal cycling protocol provided above, but subject each plate to a different cycle number (24, 25 and 26 cycles).

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

11.5 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.

11.6 Related Products

Accessory Components

PRODUCT	SIZE	CAT.#
PowerPlex® 5C Matrix Standard	5 preps	DG4850
WEN Internal Lane Standard 500 ESS	200µl	DG5101
PunchSolution™ Kit	100 preps	DC9271
SwabSolution™ Kit	100 preps	DC8271
5X AmpSolution™ Reagent	500µl	DM1231
2800M Control DNA (10ng/µl)	25µl	DD7101
2800M Control DNA (0.25ng/µl)	500µl	DD7251
Water, Amplification Grade	6,250µl (5 × 1,250µl)	DW0991

Not for Medical Diagnostic Use.

Spectrum CE System Accessories and Consumables

PRODUCT	SIZE	CAT.#
Spectrum Capillary Array, 8-Capillary	1 each	CE2008
Spectrum Polymer4	384 wells	CE2048
	960 wells	CE2040
Spectrum Buffer	2 pairs	CE2001
Spectrum Cathode Septa Mat	10 each	CE2002
Spectrum Buffer and Cathode Septa Mat Bundle	1 each	CE2012
Septa Mat, 96-Well	2 each	CE2696
Spectrum Plate Base & Retainer, 96-Well	4 each	CE5004
Spectrum Wash Solution	1 each	CE2099

Not for Medical Diagnostic Use.

[a]U.S. Pat. No. 9,139,868, European Pat. No. 2972229, Japanese Pat. No. 6367307 and other patents pending.

[b]TMR-ET, CXR-ET and WEN dyes are proprietary.

Class 1 Laser Product.