TECHNICAL MANUAL

PowerPlex® 16 BIO System

Instructions for Use of Product
DC6540

Revised 7/15
TMD016

PowerPlex® 16 BIO System

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

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1. Description

STR (short tandem repeat) loci consist of short, repetitive sequence elements 3–7 base pairs in length (1–4). These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which may be detected using the polymerase chain reaction (5–8). Alleles of STR loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using fluorescence detection following electrophoretic separation.

The PowerPlex® 16 BIO System[a–e] is used for human identification applications including forensic analysis, relationship testing and research use. The system allows coamplification and three-color detection of sixteen loci (fifteen STR loci and Amelogenin). The PowerPlex® 16 BIO System contains the loci Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818. In the system, one primer specific for Penta E, D18S51, D21S11, TH01 and D3S1358 is labeled with fluorescein (FL); one primer specific for FGA, TPOX, D8S1179, vWA and Amelogenin is labeled with Rhodamine Red™-X; and one primer specific for Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818 is labeled with 6-carboxy-4´,5´-dichloro-2´,7´-dimethoxy-fluorescein (JOE). All sixteen loci are amplified simultaneously in a single tube and analyzed in a single gel lane.

The PowerPlex® 16 Monoplex System, Penta E (Fluorescein) (Cat.# DC6591) and PowerPlex® 16 Monoplex System, Penta D (JOE) (Cat.# DC6651) are available to amplify the Penta E and Penta D loci, respectively. Each monoplex system allows amplification of a single locus to confirm results obtained with the PowerPlex® 16 System, PowerPlex® 16 BIO System or PowerPlex® 2.1 System. The monoplex systems also can be used to re-amplify DNA samples when one or more of the loci do not amplify initially due to nonoptimal amplification conditions or poor DNA quality.

The PowerPlex® 16 BIO System is designed specifically for use with the Hitachi FMBIO® II Fluorescence Imaging System. It provides all of the materials necessary to amplify STR regions of purified human genomic DNA except for AmpliTaq Gold® DNA polymerase. This manual contains separate protocols for use of the PowerPlex® 16 BIO System with the Perkin-Elmer model 480 and GeneAmp® PCR system 9600, 9700 and 2400 thermal cyclers in addition to protocols for separation and detection of amplified materials.

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

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
PowerPlex® 16 BIO System	**400 reactions**	**DC6540**

Not For Medical Diagnostic Use. Cat.# DC6540 contains sufficient reagents for 400 reactions of 25µl each. Includes:

Pre-amplification Components Box (Blue Label)
- 4 × 300µl Gold ST★R 10X Buffer
- 4 × 250µl PowerPlex® 16 BIO 10X Primer Pair Mix
- 25µl 2800M Control DNA, 10ng/µl

Post-amplification Components Box (Beige Label)
- 4 × 125µl PowerPlex® 16 BIO Allelic Ladder Mix
- 100µl Matrix 16 BIO
- 2 × 300µl Internal Lane Standard (ILS) 600 BIO
- 2 × 1ml Bromophenol Blue Loading Solution
- 250µl Gel Tracking Dye

The PowerPlex® 16 BIO Allelic Ladder Mix is provided in a separate, sealed bag for shipping. This component should be moved to the post-amplification box after opening.

Storage Conditions: Store all components except the 2800M Control DNA at –30°C to –10°C in a nonfrost-free freezer. Store the 2800M Control DNA at 2 to 10°C. The PowerPlex® 16 BIO 10X Primer Pair Mix, PowerPlex® 16 BIO Allelic Ladder Mix, Matrix 16 BIO and Internal Lane Standard 600 BIO 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.

3. Before You Begin

The application of PCR-based typing for forensic or paternity casework requires validation studies and quality-control measures that are not contained in this manual (9,10). The quality of the purified DNA, as well as 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, as well as for electrophoresis and fluorescence detection. Additional research and validation are required if any modifications are made to the recommended protocols.

PCR-based STR analysis is subject to contamination by minute amounts of human DNA. Extreme care should be taken to avoid cross-contamination when preparing sample DNA, handling primer pairs, assembling amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (Gold ST★R 10X Buffer, 2800M Control DNA and PowerPlex® 16 BIO 10X Primer Pair Mix) are provided in a separate box and should be stored separately from those used following amplification (PowerPlex® 16 BIO Allelic Ladder Mix, Internal Lane Standard 600 BIO, Matrix 16 BIO, Bromophenol Blue Loading Solution and Gel Tracking Dye). 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 (e.g., ART® tips, Section 9.G).

Some of the reagents used in the analysis of STR products are potentially hazardous and should be handled accordingly. Table 1 describes the potential hazards associated with such reagents.

Table 1. Hazardous Reagents.

Reagents	Hazards
acrylamide	suspected carcinogen, toxic
ammonium persulfate	oxidizer, corrosive
formamide (contained in the Bromophenol Blue Loading Solution)	irritant, teratogen
TEMED	corrosive, flammable
urea	irritant

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

Materials to Be Supplied by the User

- model 480 or GeneAmp® system 9600, 9700 or 2400 thermal cycler (Applied Biosystems)
- microcentrifuge
- 0.5ml or 0.2ml (thin-walled) microcentrifuge tubes, MicroAmp® optical 96-well reaction plate or MicroAmp® 8-strip reaction tubes (Applied Biosystems)
- aerosol-resistant pipette tips (see Section 9.G)
- AmpliTaq Gold® DNA polymerase (Applied Biosystems)
- Nuclease-Free Water (Cat.# P1193)
- Mineral Oil (Cat.# DY1151, for use with the thermal cycler model 480)

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

We routinely amplify 0.5–1ng of template DNA in a 25µl reaction volume using the protocols detailed below. Expect to see more intense bands for smaller loci and less intense bands for larger loci if more than the recommended amount of template is used. Reduce the amount of template DNA or the number of cycles to correct this.

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

4.A. Amplification Setup

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

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

The concentration of 2800M Control DNA was determined by measuring absorbance at 260nm. Quantification of this control DNA by other methods, such as qPCR, may result in a different value. Prepare a fresh DNA dilution for each set of amplifications. Do not store diluted DNA (e.g., 0.25ng/µl or less).

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

   Notes:

   1. Mix reagents by vortexing for 15 seconds before each use. Do not centrifuge the 10X Primer Pair Mix after vortexing, as this may cause the primers to be concentrated at the bottom of the tube.
   2. A precipitate may form in Gold ST★R 10X Buffer. If this occurs, warm the buffer briefly at 37°C, then vortex until the precipitate is in solution.

4.A. Amplification Setup (continued)

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

3. Place one clean, 0.2ml or 0.5ml reaction tube for each reaction into a rack, and label appropriately. Alternatively, use a MicroAmp® plate, and label appropriately.

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

   Table 2 shows the component volumes per reaction. A worksheet to calculate the required amount of each PCR amplification mix component is provided in Section 9.E (Table 10).

Table 2. PCR Amplification Mix for the PowerPlex® 16 BIO System.

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

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

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

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

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

5. Pipet PCR amplification mix into each reaction tube.
6. Pipet each template DNA (0.5–1ng) into the respective tube containing PCR amplification mix.
7. For the positive amplification control, vortex the tube of 2800M Control DNA; then dilute an aliquot to 0.5ng or 1ng in the desired template DNA volume. Pipet 0.5–1ng of the diluted DNA into a microcentrifuge tube containing PCR amplification mix.

   Note: To store diluted 2800M Control DNA, dilute the DNA to 0.5ng/µl in TE^{-4} buffer with 20µg/ml glycogen and store at 4°C. Do not store dilutions performed in water.
8. For the negative amplification control, pipet nuclease-free water or TE^{-4} buffer instead of template DNA into a reaction tube containing PCR amplification mix.
9. If using the GeneAmp® PCR System 9600, 9700 or 2400 thermal cycler and MicroAmp® reaction tubes or plates, no addition of mineral oil to the reaction tubes is required. However, if using the model 480 thermal cycler and GeneAmp® reaction tubes, add one drop of mineral oil to each tube before closing.

   Note: Allow the mineral oil to flow down the side of the tube and form an overlay to limit sample loss or cross-contamination due to splattering.
10. **Optional:** Briefly centrifuge the tubes to bring contents to the bottom and remove any air bubbles.

4.B. Amplification Thermal Cycling

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

Amplification and detection instrumentation may vary. Testing at Promega shows that 10/22 cycles work well for 0.5–1ng of purified DNA templates. For higher amounts of input DNA or to decrease sensitivity, fewer cycles, such as 10/16, 10/18 or 10/20, should be evaluated. In-house validation should be performed.

1. Place the tubes or MicroAmp® plate in the thermal cycler.
2. Select and run a recommended protocol. The preferred protocols for use with the GeneAmp® PCR system 9600, 9700 and 2400 and Perkin-Elmer model 480 thermal cyclers are provided below.
3. After completion of the thermal cycling protocol, store amplified samples at –20°C in a light-protected box.

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

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

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

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

The ramp rates are set in the ramp rate modification screen. While viewing the cycling program, navigate to the ramp rate modification screen by selecting "More", then "Modify". On the ramp rate modification screen the default rates for each step are 100%. The rate under each hold step is the rate at which the temperature will change to that hold temperature. Figure 1 shows the ramp rates for the GeneAmp® PCR System 9700 thermal cycler.

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

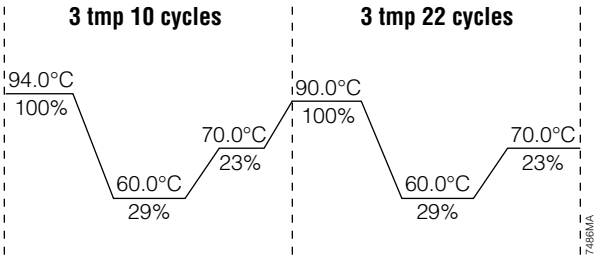

Figure 1. The ramp rates for the GeneAmp® PCR System 9700 thermal cycler.

5. Detection of Amplified Fragments Using the Hitachi FMBIO® II Fluorescence Imaging System

Materials to Be Supplied by the User

(Solution compositions are provided in Section 9.F.)

- polyacrylamide gel electrophoresis apparatus
- dry heating block, water bath or thermal cycler
- power supply (4,000 volt)
- squaretooth comb, 35cm, 60 wells (cut in half for 30 wells/gel), 0.4mm thick (Owl Scientific Cat.# S2S-60A)
- Nalgene® tissue culture filter (0.2 micron)
- aerosol-resistant pipette tips (Section 9.G)
- low-fluorescence glass plates: 43cm x 19cm x 0.4mm (The Gel Company, Cat.# GG047-B0505S)
- spacers, 0.4mm clear spacers (The Gel Company, Cat.# SGR47-036)
- SA-43 Extension (Lab Repco, Cat.# 31096423) for use with 43cm glass plates
- clamps (e.g., large office binder clamps)
- 50% Long Ranger® gel solution (Cambrex Cat.# 50611), Long Ranger Singel® pack for ABI sequencers 377–36cm (Cambrex Cat.# 50691) **or** PAGE-PLUS™ concentrate, 40% solution (Amresco, Inc., Cat.# E562)
- TBE 10X buffer
- 10% Ammonium Persulfate (Cat.# V3131)
- Urea (Cat.# V3171)
- TEMED
- bind silane (methacryloxypropyltrimethoxysilane), for use with squaretooth combs
- Liqui-Nox® detergent
- filter set for the PowerPlex® 16 BIO System (MiraiBio Cat.# 11999-246-00)

5.A. Polyacrylamide Gel Preparation

! Acrylamide (Long Ranger® gel solution) is a neurotoxin and suspected carcinogen; avoid inhalation and contact with skin. Read the warning label, and take the necessary precautions when handling this substance. Always wear gloves and safety glasses when working with acrylamide solutions.

5.A. Polyacrylamide Gel Preparation (continued)

1. Thoroughly clean glass plates twice with hot water and a 1% Liqui-Nox® solution. Rinse extremely well with deionized water. Allow the glass plates to air-dry in a dust-free environment.

   Dust particles will be detected in the scan using the 577 filter. Use lint-free paper (e.g., Kimwipes® tissues) to clean the glass plates both before pouring the gel and prior to scanning. Air from a bulb may also be used to remove dust.

   If using a squaretooth comb, one of the glass plates requires bind silane treatment. The plates do not require special silane treatment when using a sharkstooth comb.

   Bind Silane Treatment of Glass Plate
   Prepare fresh binding solution in a chemical fume hood by adding 3µl of bind silane to a 1.5ml microcentrifuge tube containing 1.0ml of 0.5% acetic acid in 95% ethanol. Wipe the short plate with a Kimwipes® tissue saturated with freshly prepared binding solution. Wait 5 minutes for the binding solution to dry. Wipe the comb area of the glass plate 5–6 times with 95% ethanol and Kimwipes® tissues to remove excess binding solution.

2. Assemble the glass plates by placing 0.4mm side spacers between the front and rear glass plates, using binder clamps to hold them in place (3–4 clamps on each side). A bottom spacer is neither required nor recommended. Place the assembly horizontally on a test tube rack or similar support.

   Note: We recommend clear spacers. If white or opaque spacers are used, place black electrical tape on the longer plate over the spacer area to prevent the spacers from being included in the scan, which can affect the signal and background.

3. Prepare a 5% Long Ranger® or 6% PAGE-PLUS™ acrylamide solution by combining the ingredients listed in Table 3.

Table 3. Preparation of 5% Long Ranger® Polyacrylamide Gels.

Component	5% Gel	Final Concentration
urea	18.0g	6M
deionized water	26.0ml	—
10X TBE Buffer	5.0ml	1X
50% Long Ranger® gel solution[1]	5.0ml	5%
total volume	**50ml**	

Component	6% PAGE-PLUS™ Gel	Final Concentration
urea	18g	6M
deionized water	24ml	—
10X TBE buffer	5ml	1X
40% PAGE-PLUS™ gel solution	7.5ml	6%
total volume	**50ml**	

[1]Long Ranger Singel® Packs may also be used.

4. Filter the acrylamide solution through a 0.2 micron filter, and pour into a squeeze bottle.
5. Add the appropriate amounts of TEMED and 10% ammonium persulfate to the acrylamide solution, and mix gently.

Component	5% Long Ranger® Gel (50ml)
TEMED	18.0g
10% ammonium persulfate	26.0ml

6. Pour the gel by starting at the well end of the plates and carefully pouring the acrylamide between the horizontal glass plates. Allow the solution to fill the top width of the plates. Slightly tilt the plates to assist movement of the solution to the bottom of the plates while maintaining a constant flow of solution. When the solution begins to flow out from the bottom, position the plates horizontally.
7. Insert the squaretooth comb between the glass plates until the teeth are almost completely inserted into the gel, or insert one 14cm doublefine (49 point) sharkstooth comb, straight side into the gel, between the glass plates (6mm of the comb should be between the two glass plates).
8. Secure the comb with three evenly spaced clamps.
9. Pour the remaining acrylamide solution into a disposable conical tube as a polymerization control. Rinse the squeeze bottle, including the spout, with water.
10. Allow polymerization to proceed for at least 1 hour. Check the polymerization control to be sure that polymerization has occurred.

    Note: The gel may be stored overnight if a paper towel saturated with deionized water and plastic wrap are placed around the top and bottom to prevent the gel from drying out (crystallization of the urea will destroy the gel).

5.B. Gel Pre-Run

1. Remove the clamps from the polymerized acrylamide gel. If necessary, clean any excess acrylamide from the glass plates with Kimwipes® tissues saturated with deionized water.
2. Shave any excess polyacrylamide away from the comb; remove the comb.
3. Add 1X TBE buffer to the bottom chamber of the electrophoresis apparatus.
4. Gently lower the gel/glass plates unit into the buffer with the longer plate facing out and the well side on top.
5. Secure the glass plates to the gel electrophoresis apparatus.
6. Add 1X TBE buffer to the top chamber of the electrophoresis apparatus.
7. Using a 50–100cc syringe filled with buffer, remove any air bubbles on the top of the gel. Be certain the well area is devoid of air bubbles and small pieces of polyacrylamide. Using a syringe with a bent 18-gauge needle, remove any air bubbles from the bottom of the gel.
8. Pre-run the gel to achieve a gel surface temperature of approximately 50°C. Consult the manufacturer's instruction manual for the recommended electrophoresis conditions.

    Note: As a reference, we generally use 60 watts for 45–60 minutes for a 43cm gel. The gel running conditions may need to be adjusted to reach a temperature of 50°C.
9. Prepare samples and allelic ladder samples during the gel pre-run.

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5.C. Sample Preparation and Loading

The Internal Lane Standard 600 (ILS) BIO is included in the PowerPlex® 16 BIO System for use as an internal size marker. With this approach, only 2–3 lanes of the PowerPlex® 16 BIO Allelic Ladder Mix are required per gel.

The Matrix 16 BIO is included with the system to aid in color separation. The Matrix 16 BIO should be run in one or two lanes per gel.

1. Prepare a loading cocktail by combining and mixing the ILS 600 BIO and Bromophenol Blue Loading Solution as follows:

	Volume Per Sample	×	Number of Lanes	=	Total Volume
Internal Lane Standard BIO	1µl	×			
Bromophenol Blue Loading Solution	3µl	×			

2. Vortex for 10–15 seconds.
3. Combine 4µl of prepared loading cocktail and 2µl of amplified sample or PowerPlex® 16 BIO Allelic Ladder Mix. Vortex the allelic ladder prior to pipetting.
4. For matrix lanes, combine 2.5µl of Matrix 16 BIO and 2.5µl of Bromophenol Blue Loading Solution.

   Note: If the fluorescent signal is too intense, dilute samples in Gold ST★R 1X Buffer before mixing with loading cocktail or use less DNA template in the amplification reactions.
5. **Optional:** Place 2µl of Gel Tracking Dye and 3µl of Gold ST★R 1X Buffer in one tube. The Gel Tracking Dye contains both bromophenol blue and xylene cyanol. This dye is loaded in the outermost lane of the gel at least 2 lanes from the nearest sample and is used as a visual indicator of migration.

   Note: The xylene cyanol dye in the Gel Tracking Dye will fluoresce when scanned with the Hitachi FMBIO® II Fluorescence Imaging System. Leave at least two empty lanes between the Gel Tracking Dye and sample lanes.
6. Briefly centrifuge samples to bring contents to the bottom of the tubes.
7. Just prior to loading the gel, denature samples by heating at 95°C for 2 minutes, and immediately chill on crushed ice or in an ice water bath.
8. After the pre-run, use a 50–100cc syringe filled with buffer and fitted with a bent 18-gauge needle to flush urea from the well area. If using a squaretooth comb, do not reinsert the comb, as the samples will be loaded directly into the wells. If using a sharkstooth comb, insert the teeth into the gel approximately 1–2mm, and leave the comb inserted in the gel during both gel loading and electrophoresis.
9. Load 3µl of each denatured sample into the respective wells. The loading process should take no longer than 20 minutes to prevent the gel from cooling.

5.D. Gel Electrophoresis

1. After loading, run the gel using the same conditions as in Section 5.B. Observe the lane containing Gel Tracking Dye to monitor sample migration. In a 5% Long Ranger® acrylamide gel, xylene cyanol dye migrates at approximately 190 bases, and in a 6% PAGE-PLUS™ acrylamide gel, xylene cyanol migrates at approximately 130 bases. In both gels, bromophenol blue migrates at less than 80 bases.
2. Based on size ranges for each locus (Table 7) and migration characteristics of the dyes contained in the Gel Tracking Dye, stop electrophoresis before the smallest locus (i.e., Amelogenin) has reached the bottom of the gel.

   Note: Some rare alleles (i.e., 9 repeats) for the D3S1358 locus are close to 100 bases in size. The gel should be stopped before the 100-base fragment of the ILS 600 BIO has reached the bottom.

Table 4. Recommended Run Times for SA-43 Gels.

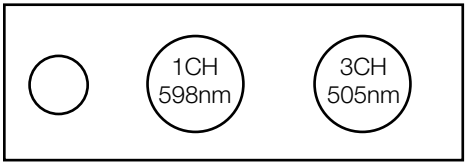
Gel Type	Leading Edge of Xylene Cyanol (distance from bottom of gel)	Approximate Run Time (60 Watts)
5% Long Ranger®	17.5cm	1 hour, 30 minutes
6% PAGE-PLUS™	9.5cm	2 hours

These recommendations are based on a one-hour pre-run. Run time and xylene cyanol migration will vary with length of pre-run.

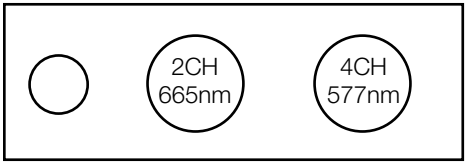
5.E. Detection

1. After electrophoresis, remove the gel/glass plate unit from the apparatus. Do not separate the glass plates.
2. The plates must be thoroughly cleaned before scanning. Thoroughly clean both sides of the gel/glass plate unit with deionized water and lint-free paper before scanning. Ethanol should not be used to clean the plate unit; ethanol fluoresces and may be detected as background by the FMBIO® instrument.
3. The protocols in the data analysis section use the following filter setup:

Filter Holder 1 (Upper Filter)



Filter Holder 2 (Lower Filter)



5.E. Detection (continued)

4. Scan the gel using the parameters listed in Table 5. Use the 598nm filter (Channel 1) to detect Rhodamine Red™-X-labeled loci, the 665nm filter (Channel 2) to detect the Texas Red®-X-labeled ILS 600 BIO, the 505nm filter (Channel 3) to detect the fluorescein-labeled fragments and the 577nm filter (Channel 4) to detect JOE-labeled fragments.

Notes:

1. Dust particles will be detected in the scan using the 577nm filter.
2. The 598nm filter in Channel 1 is acceptable for autofocusing.

Table 5. Instrument Parameters for the Hitachi FMBIO® II Fluorescence Imaging System and PowerPlex® 16 BIO System.

Parameter		Specification
Material Type		acrylamide gel
Resolution:	Horizontal	150dpi
	Vertical	150dpi
Rate		NA
Repeat		256 times
Gray Level Correction Type		range
Cutoff Threshold:	Low (background)	50%
	High (signal)	1%
Reading Sensitivity		100% (598nm channel) 100% (665nm channel) 100% (505nm channel) 100% (577nm channel)
Focusing Point[1]		0.0mm

NA = not applicable.

[1]Focusing point of 0.0mm is based on use of 5mm glass plates. If using precast gels or thinner glass plates, the focusing point may need to be adjusted. Optimal focusing point may vary from instrument to instrument.

6. Data Analysis

Four-color separation requires FMBIO® Analysis Software Macintosh® Version 8.0 or higher. The procedures included in this manual are suggestions for color separation. Further optimization may be needed for individual laboratories.

6.A. Background Adjustment

1. Create a new project using the raw data files.
2. Go to "Multi", and switch the display mode to "over".
3. Enlarge the project window to display four channels.

   Note: The initial multicolor image may not be displayed as four colors.
4. Highlight and change the colors in the boxes so that Channel 1 is red, Channel 2 is blue, Channel 3 is green and Channel 4 is yellow.
5. Perform background adjustment prior to multicolor separation. Adjust the background for each channel (Figure 2).
   a. From the multicolor image, zoom in on an area of medium-intensity band to 200X or 300X.
   b. From the multicolor image, find a medium-intensity band in the channel (color) of interest.
   c. Switch to the black and white image for that channel.
   d. From the Gray Level Adjustment window, highlight the Low (Background) percent box.
   e. Draw a box in the area directly above and almost on top of the medium-intensity band (Figure 2). Select "Set". This often results in a background above 90%.

      Note: Choose background while at 200–300X for each color.
   f. Do not adjust the signal. Signal adjustment should be performed only if the signal is unusually high. If signal needs adjustment, highlight the High (Signal) percent box, then select a band of medium intensity for signal adjustment. Select "Set".
6. Repeat Step 5 for each of the other three colors by starting at the colored image and choosing the appropriate color band.
7. Review the gel image in the Over Display Mode under "Multi". Lanes containing the Matrix 16 BIO sample should have this pattern repeated three times from the largest to smallest band: red, green, blue and yellow.

   Note: Make sure that the channels are set as shown in Step 4.
8. Perform color separation using the method described in Section 6.B or 6.C.

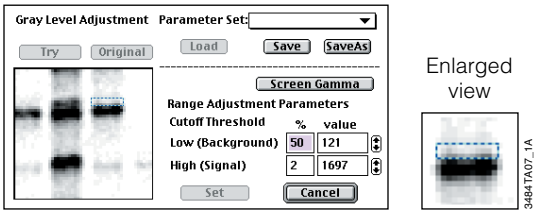


Figure 2. Area of scan to choose for background adjustment.

6.B. Color Separation Using Individual Bands

Perform the multicolor separation using individual bands from the Matrix 16 BIO lane(s). The Matrix 16 BIO sample should have this pattern repeated three times from the largest to smallest band: red, green, blue and yellow.

1. From the multicolor image, zoom in on matrix lane to 200X or 300X.
2. Highlight "Color Separation" under "Multi". A color separation window will be displayed, and the "Basis Image" shown will be red.
3. Draw a box around a red band (top band of the matrix), avoiding as much background as possible, and select "Set".
4. Repeat Steps 2 and 3 for each of the other colors by changing the "Basis Image" to the color of the band that is highlighted.
5. Select "OK" to generate the separated images.
6. Select "OK" when asked if you want to execute color separation.

6.C. Color Separation Using Bands In a Single Lane

The multicolor separation can be done using a single lane. Use the Matrix 16 BIO lane.

Read the section on image analysis tools in the FMBIO® user's manual before creating a color separation matrix with the Multi-Band Color Separator.

1. Select "1 D-Gel" under Tools.
2. Select "Layer", and highlight "filename.2CH". Select "OK".
3. Move the range to just above the top and just below the bottom of the matrix bands.
4. Select the single lane tool from the side menu, and drag it down the matrix sample lane.
5. Select "Autoband" on the side menu. Make sure that 12 bands are autobanded.
6. Select "Color Separation" from the Multi menu, then select "Multi". A Multi-Band Color Separator window will be displayed.
7. Select "Copy", or highlight a stored pattern. The pattern should have three sets of four colors with the pattern: red, green, blue and yellow.
8. If the pattern is not displayed correctly, highlight the colored box under "Use", and select the correct color.

   Note: The Matrix 16 BIO pattern can be stored by highlighting "Save as" and typing in "Matrix 16 BIO".
9. Select "OK".
10. Select "OK" from the Multi-Band Color Separator window.

6.D. Autobanding

1. View and analyze the gel image to determine allele designations as recommended in the FMBIO® user's manual. For information regarding the use of FMBIO® Analysis Software, contact the Hitachi Technical Support Group (510-337-2000 or support@miraibio.com).
2. We recommend the following autobanding parameters:

Parameter	Default Setting	Recommended Settings
Starting Slope	2.5	1.5–2.0
Ending Slope	2.5	1.5–2.0
Duration	0.2	0.1
Noise Level	20	15–30

3. The data from the four-color analysis should contain the following:

Channel/Filter	Display as (Color)	Dye Label	Loci
1/598nm	Red	Rhodamine Red™-X	FGA, TPOX, D8S1179, vWA, Amelogenin
2/665nm	Blue	Texas Red®-X	Internal Lane Standard 600 BIO
3/505nm	Green	Fluorescein	Penta E, D18S51, D21S11, TH01, D3S1358
4/577nm	Yellow	JOE	Penta D, CSF1PO, D16S539, D7S820, D13S317, D5S818

6.E. Controls

Observe the lanes containing the negative controls. Using the protocols defined in this manual, the negative controls should be devoid of amplification products.

Observe the lanes containing the 2800M Control DNA. Compare the 2800M Control DNA allelic repeat sizes with the locus-specific allelic ladder. The expected 2800M Control DNA allele designations for each locus are listed in Table 8 (Section 9.A).

6.F. Allelic Ladders

In general, allelic ladders contain fragments of the same lengths as either most or all known alleles for the locus. Allelic ladder sizes and repeat units are listed in Table 7 (Section 9.A). Visual comparison between allelic ladder and amplified samples of the same locus allows precise assignment of alleles. Analysis using specific instrumentation also allows allele determination by comparing amplified sample fragments with either allelic ladders, internal size standards or both (see software documentation from instrument manufacturer). When using an internal lane standard, the calculated lengths of allelic ladder components will differ from those listed in Table 7. This is due to differences in migration resulting from sequence differences between allelic ladder fragments and internal lane standard fragments. Dye labels also affect fragment migration.

Note: It may prove helpful to confirm that your gel analysis software identifies the correct number of alleles present in the allelic ladder lanes prior to analysis of sample lanes. The PowerPlex® 16 BIO Allelic Ladder has 86 alleles in the fluorescein channel (20 Penta E alleles, 22 D18S51 alleles, 25 D21S11 alleles, 10 TH01 alleles and 9 D3S1358 alleles), 61 alleles in the JOE channel (14 Penta D alleles, 10 CSF1PO alleles, 9 D16S539 alleles, 9 D7S820 alleles, 9 D13S317 alleles and 10 D5S818 alleles), and 55 alleles in the Rhodamine Red™-X channel (20 FGA alleles, 8 TPOX alleles, 12 D8S1179 alleles, 13 vWA alleles and 2 Amelogenin alleles).

6.G. Results

Representative results using the PowerPlex® 16 BIO System are shown in Figures 3 and 4.

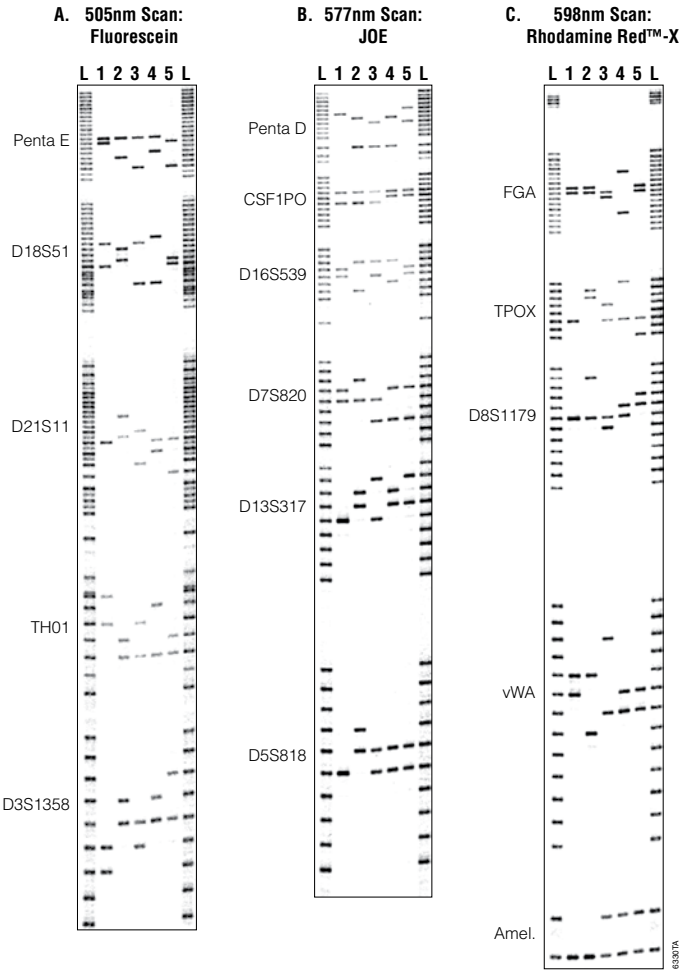


Figure 3. The PowerPlex® 16 BIO System. Five single-source DNA samples (lanes 1–5) were amplified with the PowerPlex® 16 BIO Primer Pair Mix using 1ng of DNA template. Lane 1 shows the results using 1ng of 9947A DNA. Lanes labeled L contain allelic ladders for each of the sixteen loci contained in the system. All materials were separated using a 6% PAGE-PLUS™ acrylamide gel and detected using the Hitachi FMBIO® II Fluorescence Imaging System. **Panel A.** A scan using a 505nm filter showing the fluorescein-labeled loci Penta E, D18S51, D21S11, TH01 and D3S1358. **Panel B.** A scan using a 577nm filter showing the JOE-labeled loci Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818. **Panel C.** A scan using a 598nm filter showing the Rhodamine Red™-X-labeled loci FGA, TPOX, D8S1179, vWA and Amelogenin.

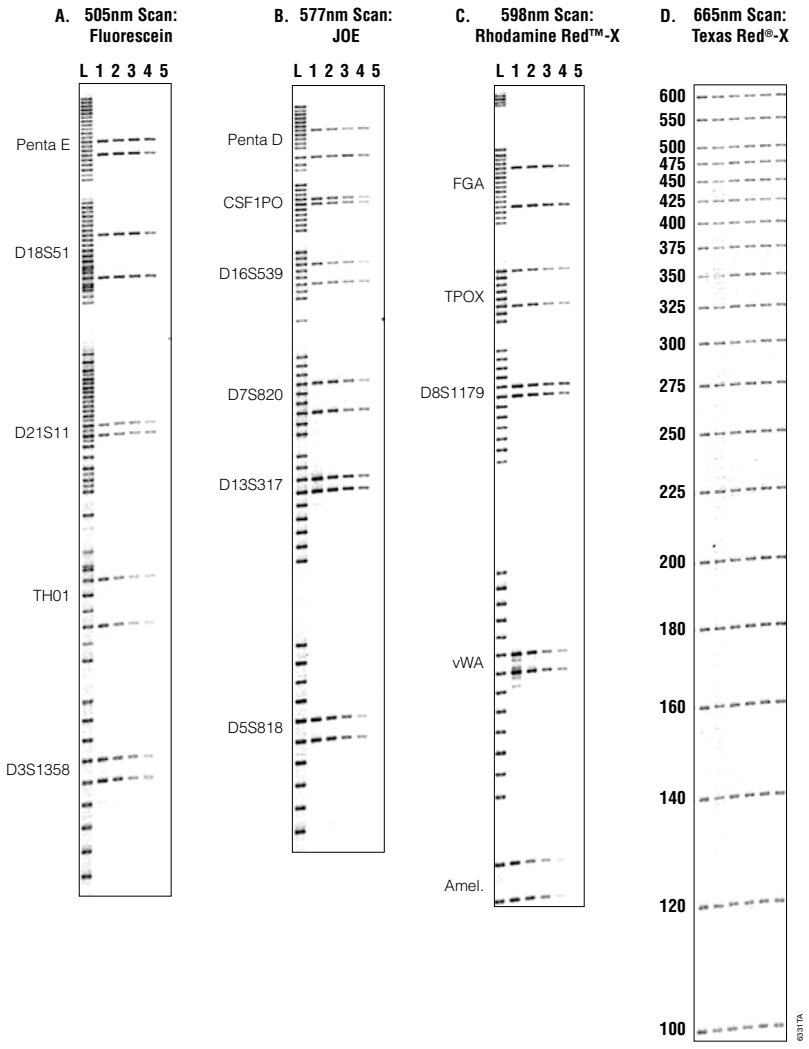


Figure 4. Amplification of various template amounts with the PowerPlex® 16 BIO System. A single-source DNA template (2, 1, 0.5 or 0.2ng) was amplified. The results are shown in lanes 1–4, respectively. Lane 5 shows the amplification results with no DNA template. Lanes labeled L contain the PowerPlex® 16 BIO Allelic Ladder. All materials were separated using a 6% PAGE-PLUS™ acrylamide gel and detected using the Hitachi FMBIO® II Fluorescence Imaging System. **Panel A.** A scan using a 505nm filter showing the fluorescein-labeled loci Penta E, D18S51, D21S11, TH01 and D3S1358. **Panel B.** A scan using a 577nm filter showing the JOE-labeled loci Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818. **Panel C.** A scan using a 598nm filter,showing the Rhodamine Red™-X-labeled loci FGA, TPOX, D8S1179, vWA and Amelogenin. **Panel D.** A scan using a 665nm filter, which results in an image of the Texas Red®-X-labeled Internal Lane Standard 600 BIO.

7. Troubleshooting

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

Symptoms	Causes and Comments
Faint or absent allele bands	Impure template DNA. Because of the small amount of template used, this is rarely a problem. Depending on the DNA extraction procedure used and sample source, inhibitors may be present in the DNA sample. Diluting the template in TE^{-4} buffer or water prior to amplification may improve results.
	Insufficient template DNA. Use the recommended amount of template DNA.
	Insufficient enzyme activity. Use the recommended amount of AmpliTaq Gold® DNA polymerase. Check the expiration date on the tube label.
	Incorrect amplification program. Confirm the amplification program.
	High salt concentration or altered pH. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the DNA volume should not exceed 20% of the total reaction volume. Carryover of K^+, Na^+, Mg^{2+} or EDTA from the DNA sample can negatively affect PCR. A change in pH also may affect PCR. Store DNA in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or nuclease-free water.
	Primer concentration was too low. Use the recommended primer concentration. Vortex the PowerPlex® 16 BIO 10X Primer Pair Mix for 15 seconds before use.
	Thermal cycler, plate or tube problems. Review the thermal cycling protocols in Section 4.B. We have not tested other reaction tubes, plates or thermal cyclers. Calibrate the thermal cycler heating block if necessary.
	Samples were not denatured completely. Heat-denature samples at 95°C for 2 minutes, and immediately chill on crushed ice or in an ice water bath prior to loading the gel. Do not cool the samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing.
	Background level was too high (≥99%) in the black and white image in question. Repeat the color separation process starting with the gray level adjustment and using raw data, **OR** lower the background to a percentage equivalent to the percentage that was set in the other three images.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
Bands are fuzzy throughout the lanes	Poor-quality polyacrylamide gel. Prepare acrylamide and buffer solutions using high-quality reagents. We recommend 5% Long Ranger® or 6% PAGE-PLUS™ gels.
	Gel pre-run was not long enough. Pre-run the gel until the gel temperature is 50°C.
	Electrophoresis temperature was too high. Run gel at lower temperature (40–50°C).
(n–1) bands present	Following amplification, lengthen the final extension step from 30 minutes at 60°C to 45 minutes. n–1 bands may be generated when more than 1ng of template DNA is used. This is most commonly observed with the vWA and D3S1358 amplification products. Reduce the amount of template DNA used, or reduce the number of cycles by 2 or 4 cycles (10/20 or 10/18 cycles).
Incorrect matrix pattern	Incorrect filter set was used or filters in the wrong position for scanning. Filters for scanning should be: Position 1: 598 Position 2: 665 Position 3: 505 Position 4: 577
	Incorrect colors were assigned to the channels. Channel colors should be: Channel 1: red Channel 2: blue Channel 3: green Channel 4: yellow
Extra bands 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.
	Artifacts of STR amplification. Amplification of STRs sometimes generates artifacts that appear as faint peaks one repeat unit smaller than the allele. Stutter band peak heights will be high if the samples are overloaded.
	Allelic ladder contamination. Store pre- and post-amplification components separately.
	Samples were not completely denatured. Heat-denature samples at 95°C for 2 minutes, and immediately chill on crushed ice or in an ice-water bath prior to loading the gel. Do not cool the samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing.

Symptoms	Causes and Comments
Bleedthrough	Incorrect gray level. Use matrix lanes for color separation. Confirm that the bands selected are from the appropriate scanning channel.
	Incorrect colors were assigned to the channels. Repeat gray level adjustment (Figure 2).
	Signal was too strong. Use less template in the amplification reactions, or load less amplified sample onto the gel.
	Gel was not run long enough. Bands become more diffuse (less intense) during the gel run. Gels should be run until the 100-base fragment is near the bottom of the gel.
Imbalance of band intensities across loci	Too much template. The system is balanced using 1ng of DNA template. Using greater than 2ng will lead to overrepresented smaller alleles and underrepresented larger alleles. Use the recommended amount of template. Alternatively, reduce the number of cycles in the amplification program to improve locus-to-locus balance.
	Poor-quality polyacrylamide gel. Prepare acrylamide and buffer solutions using high-quality reagents. We recommend 5% Long Ranger® or 6% PAGE-PLUS™ gels.
	Too many cycles in the amplification protocol. Use the recommended amplification program; confirm the number of cycles.
	Too much enzyme present. Use the recommended amount of AmpliTaq Gold® DNA polymerase.
	Degraded DNA sample. Confirm the DNA integrity by running an aliquot on an agarose gel. Repurify the template DNA if necessary.
	Miscellaneous balance problems. Thaw the 10X Primer Pair Mix and Gold ST★R 10X Buffer completely, and vortex for 15 seconds before using. Calibrate thermal cyclers and pipettes routinely. Decreasing the annealing temperatures by 1–2°C also can improve the balance.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
Imbalance of band intensities across loci (continued)	Too much template from card or membrane punches of bloodstains. Cards or membranes that bind DNA tightly can contain more template DNA than recommended. This will lead to overrepresented smaller alleles and underrepresented larger alleles. Use the recommended amount of template by using a smaller punch of the membrane. Alternatively, fewer cycles of amplification can compensate for this type of unevenness of product yield (i.e., 10/20 or 10/18 cycling).
	Incorrect plates or tubes were used. Use only MicroAmp® tubes or plates. Due to potential differences in heat transfer capabilities, plates or tubes from other manufacturers might result in imbalance.
Poor separation of alleles in ladder lanes or difficulty resolving microvariant alleles	Gel was not run long enough. Run gel as long as possible without running the 100-base ILS fragment off the bottom of the gel. If necessary run the gel for additional time after the first scan, then scan the gel a second time to achieve greater separation of larger alleles.
	Scanning resolution was too low. Default scanning resolution is 150dpi. If necessary this resolution can be increased to 300dpi, which should help sharpen bands.
White background with low signal intensity	Part of the white spacers were scanned. Rescan the gel, being careful not to scan any portion of the spacers, remove the spacers for scanning or use black electrical tape to cover the spacers. We recommend using clear spacers.
Dark, grainy background with low signal intensity	Focusing point may need to be adjusted. Perform multiple scans using different focusing points. Adjust focusing point if necessary.
	Plates were improperly washed. Improper washing of plates can cause a soap residue to build up on the plates, which can cause background fluorescence. Plate may be soaked in 1N NaOH to remove residue. Do not use ethanol to clean plates prior to scanning.

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Additional STR references can be found at: **www.promega.com/geneticidentity/**

9. Appendix

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

The loci included in the PowerPlex® 16 BIO System (Tables 6, 7 and 8) were selected because they satisfy the needs of several major standardization bodies throughout the world. For example, the United States Federal Bureau of Investigation (FBI) has selected 13 STR core loci for typing prior to searching or including (submitting) samples in the Combined DNA Index System (CODIS), the U.S. national database of convicted offender profiles. The PowerPlex® 16 BIO System amplifies all CODIS core loci in a single reaction.

The PowerPlex® 16 BIO System also contains two low-stutter, highly polymorphic pentanucleotide repeat loci, Penta E and Penta D. These additional loci add significantly to the discrimination power of the system, making the PowerPlex® 16 BIO System a single-amplification system with a power of exclusion sufficient to resolve paternity disputes definitively. In addition, the extremely low stutter seen with Penta E and Penta D makes them ideal loci to evaluate DNA mixtures often encountered in forensic casework. Finally, the Amelogenin locus is included in the PowerPlex® 16 BIO System to allow gender identification of each sample. Table 8 lists the system alleles revealed in commonly available standard DNA templates.

We have carefully selected STR loci and primers to avoid or minimize artifacts, including those associated with *Taq* DNA polymerase, such as repeat slippage and terminal nucleotide addition. Repeat slippage (11,12), sometimes called "n–4 bands," "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 DNA sequence being amplified.

Terminal nucleotide addition (13,14) occurs when *Taq* DNA polymerase adds a nucleotide, generally adenine, to the 3´ ends of amplified DNA fragments in a template-independent manner. The efficiency with which this occurs varies with different primer sequences. Thus, an artifact band one base shorter than expected (i.e., missing the terminal addition) is sometimes seen. We have modified primer sequences and added a final extension step of 60°C for 30 minutes (15) to the amplification protocol to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of DNA template are used.

The presence of microvariant alleles (alleles differing from one another by lengths other than the repeat length) complicates interpretation and assignment of alleles. There appears to be a correlation between a high degree of polymorphism, a tendency for microvariants and increased mutation rate (16,17). Thus, FGA and D21S11 display numerous, relatively common microvariants. For reasons yet unknown, the highly polymorphic Penta E locus does not display frequent microvariants (Table 7).

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Table 6. The PowerPlex® 16 BIO System Locus-Specific Information.

STR Locus	Label	Chromosomal Location	GenBank® Locus and Locus Definition	Repeat Sequence[1] 5´→ 3´
Penta E	FL	15q	NA	AAAGA
D18S51	FL	18q21.3	HUMUT574	AGAA (18)
D21S11	FL	21q11–21q21	HUMD21LOC	TCTA Complex (18)
TH01	FL	11p15.5	HUMTH01, Human tyrosine hydroxylase gene	AATG (18)
D3S1358	FL	3p	NA	TCTA Complex
FGA	Rhodamine Red™-X	4q28	HUMFIBRA, Human fibrinogen alpha chain gene	TTTC Complex (18)
TPOX	Rhodamine Red™-X	2p24–2pter	HUMTPOX, Human thyroid peroxidase gene	AATG
D8S1179	Rhodamine Red™-X	8q	NA	TCTA Complex (18)
vWA	Rhodamine Red™-X	12p12–pter	HUMVWFA31, Human von Willebrand factor gene	TCTA Complex (18)
Amelogenin[2]	Rhodamine Red™-X	Xp22.1–22.3 and Y	HUMAMEL, Human Y chromosomal gene for Amelogenin-like protein	NA
Penta D	JOE	21q	NA	AAAGA
CSF1PO	JOE	5q33.3–34	HUMCSF1PO, Human c-fms proto-oncogene for CSF-1 receptor gene	AGAT
D16S539	JOE	16q24–qter	NA	GATA
D7S820	JOE	7q11.21–22	NA	GATA
D13S317	JOE	13q22–q31	NA	TATC
D5S818	JOE	5q23.3–32	NA	AGAT

[1]The August 1997 report (19,20) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, "1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5´ nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used".

[2]Amelogenin is not an STR but displays a 106-base, X-specific band and a 112-base, Y-specific band.

FL = fluorescein.

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

NA = not applicable.

Table 7. The PowerPlex® 16 BIO System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components[1,2] (bases)	Repeat Numbers of Allelic Ladder Components	Repeat Numbers of Alleles Not Present in Allelic Ladder[3,4]
Penta E	FL	379–474	5–24	20.3
D18S51	FL	290–366	8–10, 10.2, 11–13, 13.2, 14–27	
D21S11	FL	203–259	24, 24.2, 25, 25.2, 26–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38	
TH01	FL	156–195	4–9, 9.3, 10–11, 13.3	
D3S1358	FL	115–147	12–20	
FGA	Rhodamine Red™-X	322–444	16–30, 31.2, 43.2, 44.2, 45.2, 46.2	18.2, 19.2, 22.2, 23.2, 24.2, 25.2
TPOX	Rhodamine Red™-X	262–290	6–13	
D8S1179	Rhodamine Red™-X	203–247	7–18	
vWA	Rhodamine Red™-X	123–171	10–22	
Amelogenin[5]	Rhodamine Red™-X	106, 112	X, Y	
Penta D	JOE	376–449	2.2, 3.2, 5, 7–17	
CSF1PO	JOE	321–357	6–15	
D16S539	JOE	264–304	5, 8–15	
D7S820	JOE	215–247	6–14	
D13S317	JOE	176-208	7–15	
D5S818	JOE	119–155	7–16	

[1]Lengths of each allele in the allelic ladders have been confirmed by sequence analyses.

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

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

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

[5]Amelogenin is not an STR but displays a 106-base, X-specific band and a 112-base, Y-specific band.

Table 8. The PowerPlex® 16 BIO System Allele Determinations in Commonly Available Standard DNA Templates.

	Standard DNA Templates[1]			
STR Locus	**K562[2]**	**9947A**	**9948[3]**	**2800M**
Penta E	5, 14	12, 13	11, 11	7, 14
D18S51	15, 16	15, 19	15, 18	16, 18
D21S11	29, 30, 31	30, 30	29, 30	29, 31.2
TH01	9.3, 9.3	8, 9.3	6, 9.3	6, 9.3
D3S1358	16, 16	14, 15	15, 17	17, 18
FGA	21, 24	23, 24	24, 26	20, 23
TPOX	8, 9	8, 8	8, 9	11, 11
D8S1179	12, 12	13, 13	12, 13	14, 15
vWA	16, 16	17, 18	17, 17	16, 19
Amelogenin	X, X	X, X	X, Y	X, Y
Penta D	9, 13	12, 12	8, 12	12, 13
CSF1PO	9, 10	10, 12	10, 11, 12	12, 12
D16S539	11, 12	11, 12	11, 11	9, 13
D7S820	9, 11	10, 11	11, 11	8, 11
D13S317	8, 8	11, 11	11, 11	9, 11
D5S818	11, 12	11, 11	11, 13	12, 12

[1]Information on strains K562, 9947A and 9948 is available online at: **http://ccr.coriell.org**
Strain K562 is available from the American Type Culture Collection: **www.atcc.org** (Manassas, VA).
Information about the use of 9947A and 9948 DNA as standard DNA templates can be found in reference 21.

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

[3]Strain 9948 displays three alleles at the CSF1PO locus. The peak height for allele 12 is much lower than those for alleles 10 and 11.

9.B. Power of Discrimination

The fifteen STR loci amplified with the PowerPlex® 16 BIO System provide powerful discrimination. Population statistics for these loci and their various multiplex combinations are displayed in Table 9. These data were developed as part of a collaboration (22) with The Bode Technology Group (Springfield, VA), North Carolina Bureau of Investigation (Raleigh, NC), Palm Beach County Sheriff's Office (West Palm Beach, FL), Virginia Division of Forensic Science (Richmond, VA) and Charlotte/ Mecklenburg Police Department Laboratory (NC). Generation of these data includes analysis of over 200 individuals from African-American, Caucasian-American and Hispanic-American populations. Data for Asian-Americans includes analysis of over 150 individuals. For additional population data for STR loci, see references 23–28 and the Short Tandem Repeat DNA Internet DataBase at: **www.cstl.nist.gov/div831/strbase/**

Table 9 shows the matching probability (29) for the PowerPlex® 16 BIO System in various populations. The matching probability of this system ranges from 1 in 1.83×10^{17} for Caucasian-Americans to 1 in 1.41×10^{18} for African-Americans.

A measure of discrimination often used in paternity analyses is the paternity index (PI), a means for presenting the genetic odds in favor of paternity given the genotypes for the mother, child and alleged father. The typical paternity indices for the PowerPlex® 16 BIO System are shown in Table 9. This system provides typical paternity indices exceeding 500,000 in each population group. An alternative calculation used in paternity analyses is the power of exclusion (30). This value, calculated for the system, exceeds 0.999998 in all populations tested (Table 9).

Table 9. Matching Probabilities, Paternity Indices and Power of Exclusion of the PowerPlex® 16 BIO System in Various Populations.

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

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

The DNA IQ™ System (Cat.# DC6700) is a DNA isolation system designed specifically for forensic and paternity samples (31). This novel system uses paramagnetic particles to prepare clean samples for STR analysis easily and efficiently and can be used to extract DNA from stains or liquid samples, such as blood or solutions. The DNA IQ™ Resin eliminates PCR inhibitors and contaminants frequently encountered in casework samples. With larger samples, the DNA IQ™ System delivers a consistent amount of total DNA. The system has been used to isolate and quantify DNA from routine sample types including buccal swabs, stains on FTA® paper and liquid blood. Additionally, DNA has been isolated from casework samples such as tissue, differentially separated sexual assault samples and stains on support materials. The DNA IQ™ System has been tested with the PowerPlex® Systems to ensure a streamlined process. See Section 9.G for ordering information.

For applications requiring human-specific DNA quantification, the Plexor® HY System (Cat.# DC1001, DC1000) has been developed (32). See Section 9.G for ordering information.

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

9.D. The Internal Lane Standard 600 BIO

The Internal Lane Standard (ILS) 600 BIO contains 21 DNA fragments of 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases in length. Each fragment is labeled with Texas Red®-X and may be detected separately (as a fourth color) in the presence of PowerPlex® 16 BIO amplified material using the Hitachi FMBIO® II Fluorescence Imaging System. The ILS 600 BIO is designed for use in each gel lane to increase precision in analyses when using the PowerPlex® 16 BIO System. A protocol for preparation and use of this internal lane standard is provided in Section 5.C.

9.E. Preparing the PowerPlex® 16 BIO System PCR Amplification Mix

Use Table 10 to calculate the required amount of each component of the PCR amplification mix. Multiply the volume (µl) per sample by the total number of reactions to obtain the final PCR amplification mix volume (µl).

Table 10. PCR Amplification Mix for the PowerPlex® 16 BIO System.

PCR Master Mix Component	Volume Per Sample	×	Number of Reactions	=	Final Volume (µl)
Gold ST★R 10X Buffer	2.5µl	×		=	
PowerPlex® 16 BIO 10X Primer Pair Mix	2.5µl	×		=	
AmpliTaq Gold® DNA polymerase[1]	0.8µl (4u)	×		=	
nuclease-free water[2]	µl	×		=	
		×		=	
Per tube					
template DNA volume[2] (0.5–1ng)	up to 19.2µl	×		=	
total reaction volume	**25µl**	×		=	

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

[2]The PCR amplification mix volume added to the template DNA volume should total 25µl. Consider the volume of template DNA, and add nuclease-free water to the PCR amplification mix to bring the final volume of the final reaction to 25µl.

9.F. Composition of Buffers and Solutions

10% ammonium persulfate

Add 0.05g of ammonium persulfate to 500µl of deionized water.

Bromophenol Blue Loading Solution

10mM	NaOH
95%	formamide
0.05%	bromophenol blue

Gel Tracking Dye

10mM	NaOH
95%	formamide
0.05%	bromophenol blue
0.05%	xylene cyanol FF

Gold ST★R 10X Buffer

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

TBE 10X buffer

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

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

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

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

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

TE^{-4} buffer with 20µg/ml glycogen

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

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

9.G. Related Products

Product	Size	Cat.#
PowerPlex® 16 Monoplex System, Penta E (Fluorescein)	100 reactions	DC6591
PowerPlex® 16 Monoplex System, Penta D (JOE)	100 reactions	DC6651

Not For Medical Diagnostic Use.

Accessory Components

Product	Size	Cat.#
2800M Control DNA*	25µl	DD7101
	500µl	DD7251
Gold ST★R 10X Buffer	1.2ml	DM2411
Nuclease-Free Water	50ml	P1193

*Not For Medical Diagnostic Use.

Sample Preparation Systems

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

*Not for Medical Diagnostic Use.

Polyacrylamide Gel Electrophoresis Reagents

Product	Size	Cat.#
Ammonium Persulfate, Molecular Grade	25g	V3131
TBE Buffer, 10X, Molecular Biology Grade	1L	V4251
Urea	1kg	V3171

9.H. Summary of Changes

The following changes were made to the 7/15 revision of this document:

1. The patent/license statements were updated.
2. The discontinued 100-reaction size of the product was removed.
3. The document design was updated.

[a]PowerPlex® 16 BIO System incorporates dye conjugates made with the Rhodamine Red™-X and Texas Red®-X fluorescent reactive dyes, which are licensed from Molecular Probes, Inc., under U.S. Pat. Nos. 5,798,276 and 5,846,737 for DNA analysis. Rhodamine Red is a trademark of Molecular Probes, Inc., and Texas Red is a registered trademark of Molecular Probes, Inc.

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

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

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

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

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All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.