



Promega

Technical Manual

PowerPlex® 1.1 System

INSTRUCTIONS FOR USE OF PRODUCTS DC6091, DC6090, DG2341,
DG6221 AND DC6171



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Part# TMD008

PowerPlex® 1.1 System



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Please visit the web site to verify that you are using the most current version of this Technical Manual.
Please contact Promega Technical Services if you have questions on use of this system.
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1. Description

STR^(a) (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 that can be detected using PCR (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 radioactive, silver stain or fluorescence detection following electrophoretic separation.

The PowerPlex[®] 1.1 System^(a,b) allows the co-amplification and two-color detection of eight STR loci. In the PowerPlex[®] 1.1 System, one of the two primers for D16S539, D7S820, D13S317 and D5S818 is labeled with fluorescein (FL), and one primer specific for CSF1PO, TPOX, TH01 and vWA is labeled with carboxy-tetramethyl-rhodamine (TMR). All eight loci are amplified simultaneously in a single tube and analyzed in a single gel lane. Loci amplified using the PowerPlex[®] 1.1 System also can be co-amplified with the *GenePrint*[®] Fluorescent Sex Identification System – Amelogenin (TMR), allowing the detection of **nine loci** in a single gel lane.

The PowerPlex[®] 1.1 System is customized for use with the Hitachi FMBIO[®] and FMBIO[®] II fluorescence imaging systems. The PowerPlex[®] 1.2 System is optimized for use with the ABI PRISM[®] 310 genetic analyzer and is also compatible with the ABI PRISM[®] 377 DNA sequencer.

The PowerPlex[®] 1.1 System provides all of the materials necessary for amplification of STR regions of purified genomic DNA except for *Taq* DNA polymerase. This manual contains protocols for use of the PowerPlex[®] 1.1 System with the Perkin-Elmer model 480 and GeneAmp[®] PCR system 9600 thermal cyclers in addition to protocols for separation of amplified products and detection of separated material. Protocols for operation of the fluorescence-detection instrumentation should be obtained from the manufacturer.

Information on other Promega fluorescent STR systems and detection of amplified STR fragments using silver staining is available upon request from Promega or online at: www.promega.com/geneticidentity/

2. Product Components and Storage Conditions



Product	Size	Cat.#
PowerPlex® 1.1 System	100 reactions	DC6091

Not For Medical Diagnostic Use. Cat.# DC6091 contains sufficient reagents for 100 reactions of 25µl each. Includes:

- 300µl STR 10X Buffer
- 250µl PowerPlex® 1.1 10X Primer Pair Mix
- 150µl PowerPlex® 1.1 Allelic Ladder Mix
- 25µl *GenePrint*® TH01 Allele 9.3 (TMR)
- 125µl Fluorescent Ladder (CXR), 60–400 Bases
- 3µg K562 DNA High Molecular Weight (10ng/µl)
- 1ml Bromophenol Blue Loading Solution
- 250µl Gel Tracking Dye

Product	Size	Cat.#
PowerPlex® 1.1 System	400 reactions	DC6090

Not For Medical Diagnostic Use. Cat.# DC6090 contains sufficient reagents for 400 reactions of 25µl each. Includes:

- 4 × 300µl STR 10X Buffer
- 4 × 250µl PowerPlex® 1.1 10X Primer Pair Mix
- 4 × 150µl PowerPlex® 1.1 Allelic Ladder Mix
- 100µl *GenePrint*® TH01 Allele 9.3 (TMR)
- 4 × 125µl Fluorescent Ladder (CXR), 60–400 Bases
- 3µg K562 DNA High Molecular Weight (10ng/µl)
- 2 × 1ml Bromophenol Blue Loading Solution
- 250µl Gel Tracking Dye

Storage Conditions: Store all components at -20°C in a nonfrost-free freezer. The 10X Primer Pair Mix, Allelic Ladder Mix and Fluorescent Ladder (CXR) are light-sensitive and must be stored in the dark. The post-amplification components (Allelic Ladder Mix, Fluorescent Ladder (CXR), Bromophenol Blue Loading Solution and Gel Tracking Dye) are sealed in separate packages to prevent cross-contamination. We strongly recommend that pre-amplification and post-amplification reagents be stored and used separately with different pipettes, tube racks, etc.

Product	Size	Cat.#
PowerPlex® 1.1 Allelic Ladder Mix*	150µl	DG2341
<i>GenePrint</i> ® Fluorescent Sex Identification System – Amelogenin (TMR)*	100 reactions	DC6171

*Not For Medical Diagnostic Use.

Additional fluorescent STR multiplex product information and ordering information for accessory components and related products is provided in Section 9.J.

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 sample, as well as small changes in buffers, ionic strength, primer concentrations, choice of thermal cycler and thermal cycling conditions can affect the success of a PCR amplification. We suggest strict adherence to recommended procedures for amplification, as well as for electrophoresis and fluorescence detection.

PCR-based STR analysis is subject to contamination by very small amounts of nontemplate 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 (STR 10X Buffer, K562 DNA and PowerPlex® 1.1 10X Primer Pair Mix) should be stored separately from those used following amplification (PowerPlex® Allelic Ladder Mix, Fluorescent Ladder (CXR), Bromophenol Blue Loading Solution and Gel Tracking Dye). Always include a negative control reaction (i.e., no template) to ensure reagent purity. We highly recommend the use of gloves and aerosol resistant pipette tips (e.g., ART® tips, Section 9.J).

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.


Reagent	Hazard
acrylamide	suspected carcinogen, toxic
ammonium persulfate	oxidizer, corrosive
bisacrylamide	toxic, irritant
formamide (contained in the Bromophenol Blue Loading Solution and Gel Tracking Dye)	irritant, teratogen
TEMED	corrosive, flammable
urea	irritant

4. Protocols for DNA Amplification Using the PowerPlex® 1.1 System

Materials to Be Supplied by the User

- thermal cycler 480 or GeneAmp® PCR System 9600
- microcentrifuge
- GeneAmp® 0.5ml tubes, MicroAmp® reaction tubes or MicroAmp® optical 96-well reaction plate (Applied Biosystems)
- 1.5ml amber-colored microcentrifuge tubes (Fisher Cat.# 05-402-26)
- aerosol-resistant pipette tips (see Section 9.J)
- *Taq* DNA polymerase
- Nuclease-Free Water (Cat.# P1193)
- Mineral Oil (Cat.# DY1151, for use with the model 480 thermal cycler)

We routinely amplify 1-2ng of template DNA in a 25µl reaction volume using the protocols detailed below. Amplification protocols for both the DNA thermal cycler 480 and the GeneAmp® PCR system 9600 thermal cycler are provided.

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

The PowerPlex® 1.1 System has been developed for amplification without artifacts using standard *Taq* DNA polymerase. Special enzymes such as AmpliTaq Gold® DNA polymerase are not required for peak performance. However, **if using AmpliTaq Gold® DNA polymerase, we recommend using the Gold ST*R 10X Buffer (Cat.# DM2411, available separately) instead of the STR 10X Buffer.** The STR 10X Buffer (pH 9.0) is not compatible with AmpliTaq Gold® DNA polymerase because the optimal pH for the modified *Taq* DNA polymerase is 8.3. Also, when using AmpliTaq Gold® DNA polymerase, an additional incubation at 95°C for 11 minutes must be incorporated prior to initiation of the thermal cycling program.

4.A. Precautions

1. Prepare amplifications in a designated clean room away from any post-amplification samples and allelic ladders.
2. Designate pipettes, tips and racks to be used for all pre-amplification tasks.
3. To prevent cross-contamination, use aerosol-resistant pipette tips (see Section 9.J).
4. Wear gloves and a lab coat to avoid contamination. Change gloves often.
5. Use sterile tubes for all master mixes and reactions.
6. Keep master mix and all tubes on ice during setup. If using AmpliTaq Gold® DNA polymerase, it is not necessary to keep the reactions on ice.

4.B. Amplification Setup

1. Thaw the STR 10X Buffer and PowerPlex® 1.1 10X Primer Pair Mix, and **store on ice**. It is very important to mix both reagents by vortexing for 15 seconds before each use.
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 waste a small amount of each reagent, it ensures that you will have enough PCR master mix for all samples. It also ensures that each reaction contains the same master 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.

Note: If using the GeneAmp® PCR system 9600 thermal cycler, use 0.2ml thin-walled MicroAmp® reaction tubes or MicroAmp® plate. For the PE Biosystems thermal cycler model 480, use 0.5ml thin-walled GeneAmp® reaction tubes.

4. Add the volume of each reagent listed in Table 2 or 3 into a sterile, 1.5ml amber-colored tube. Mix gently.

Tables 2 and 3 show the component volumes per reaction without (Table 2) and with (Table 3) co-amplification of Amelogenin. Worksheets to calculate the required amount of each component of the PCR master mix are provided in Section 9.G (Tables 13 and 14).

Note: The PowerPlex® 1.1 System and the *GenePrint*® Fluorescent Sex Identification System – Amelogenin (TMR) (Cat.# DC6171) can be amplified simultaneously for a total of nine loci (Table 3). **Do not use the fluorescein-labeled Sex Identification System – Amelogenin (Cat.# DC5171) with the PowerPlex® 1.1 System.**

Optional: If desired, BSA Fraction V (final concentration 160µg/ml) may be added to the reactions. Performance may vary depending on the source of this component.

5. In the order listed in Tables 2 and 3, add the final volume of each reagent into a sterile, 1.5ml amber-colored tube. Mix gently (do not vortex), and **place on ice**.

Note: If the volume of *Taq* DNA polymerase added to the master mix is less than 0.5µl, you may wish to dilute the enzyme with STR 1X Buffer first and add a larger volume. The amount of Nuclease-Free Water in the reaction should be adjusted accordingly so that the final volume of master mix per reaction is 22.5µl. Do not store diluted *Taq* DNA polymerase.

6. Pipet PCR master mix into each reaction tube or well, and **place on ice**.

Note: **Failure to keep the reagents and samples on ice can produce imbalanced amplification of multiplexed loci.** If using AmpliTaq Gold® DNA polymerase, it is not necessary to keep the reactions on ice.

7. Pipet the template DNA (1–2ng) for each sample into the respective tube containing PCR master mix.

Table 2. PCR Master Mix for the PowerPlex® 1.1 System.

PCR Master Mix Component ¹	Volume Per Sample
Nuclease-Free Water	to a final volume of 25.0µl
STR 10X Buffer	2.5µl
PowerPlex® 1.1 10X Primer Pair Mix	2.5µl
<i>Taq</i> DNA polymerase ²	0.4µl (2u)
template DNA (1-2ng) ³	up to 19.6µl
total reaction volume	25µl

Table 3. PCR Master Mix for the PowerPlex® 1.1 System and Amelogenin (TMR).

PCR Master Mix Component ¹	Volume Per Sample
Nuclease-Free Water	to a final volume of 25.0µl
STR 10X Buffer	2.5µl
PowerPlex® 1.1 10X Primer Pair Mix	2.5µl
Amelogenin 10X Primer Pair (TMR)	2.5µl
<i>Taq</i> DNA polymerase ²	0.45µl (2.25u)
template DNA (1-2ng) ³	up to 17.05µl
total reaction volume	25µl

¹Add nuclease-free water to the PCR master mix first, then add STR 10X Buffer, PowerPlex® 1.1 10X Primer Pair Mix and *Taq* DNA polymerase. The template DNA will be added at Step 7.

²Assumes that the *Taq* DNA polymerase is at a concentration of 5u/µl. If the enzyme concentration is different, the volume of enzyme used must be adjusted accordingly.

³Store 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 the DNA sample added should not exceed 20% of the final reaction volume. PCR amplification efficiency and quality can be altered greatly 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.

8. For the **positive** amplification control, dilute the K562 DNA to 0.4–0.8ng/µl. Pipet 2.5µl (1–2ng) of diluted K562 DNA into a tube or well containing 22.5µl of PCR master mix.
9. For the **negative** amplification control, pipet 2.5µl of Nuclease-Free Water (instead of template DNA) into a tube or well containing 22.5µl of the PCR master mix.
10. If using the GeneAmp® PCR System 9600 thermal cycler and MicroAmp® reaction tubes or plates, no addition of mineral oil is required. However, if using the model 480 thermal cycler and GeneAmp® reaction tubes, add 1 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.

11. Centrifuge the samples briefly to bring the aqueous contents to the bottom of the tube or well.

4.C. Amplification Thermal Cycling

This manual contains protocols for use of the PowerPlex® 1.1 System with the Perkin-Elmer model 480 and GeneAmp® PCR system 9600 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. You may need to optimize protocols including cycle number and injection time (or loading volume) for each laboratory instrument. 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 Perkin-Elmer model 480 and the GeneAmp® system 9600 thermal cyclers are provided below.

Protocol for the Perkin-Elmer Model 480 Thermal Cycler

When using AmpliTaq Gold® DNA polymerase, incorporate an additional incubation at 95°C for 11 minutes prior to initiation of thermal cycling.

Cycling profile: 96°C for 2 minutes, then:
94°C for 1 minute
60°C for 1 minute
70°C for 1.5 minutes
For **10 cycles**, then:
90°C for 1 minute
60°C for 1 minute
70°C for 1.5 minutes
For **20 cycles**, then:
60°C for 30 minutes

Protocol for the Perkin-Elmer GeneAmp® PCR System 9600 Thermal Cycler

Note: We generally use 0.2ml thin-walled MicroAmp® reaction tubes with this thermal cycler. When using AmpliTaq Gold® DNA polymerase, incorporate an additional incubation at 95°C for 11 minutes prior to initiation of thermal cycling.

Cycling profile: 96°C for 1 minute, 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:
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 **20 cycles**, then:
60°C for 30 minutes

3. After completion of the thermal cycling protocol, store the samples at -20°C in a light-protected box.

Note: Storage of amplified samples at 4°C or higher may produce degradation products.

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

Materials to Be Supplied by the User

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

- polyacrylamide gel electrophoresis apparatus
- power supply (4,000 volt)
- dry heating blocks (2) or water baths (2)
- square-tooth comb, 35cm, 60 wells (cut in half for 30wells/gel), 0.4mm thick (Owl Scientific Cat.# S2S-60A)
- vinyl doublefine sharktooth comb(s), 14cm, 49 point, 0.4mm thick
- Nalgene® tissue culture filter (0.2 micron)
- aerosol-resistant pipette tips (Section 9.J)
- low-fluorescence glass plates: 43cm × 19cm × 0.4mm (height × width × thickness) (The Gel Company Cat.# GG047-B0505S)
- gel 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)
- diamond pencil for marking glass plates
- 40% acrylamide:bis (19:1) and TEMED
- TBE 10X buffer
- 10% ammonium persulfate
- urea
- Liqui-Nox® detergent
- crushed ice or an ice-water bath

5.A. Polyacrylamide Gel Preparation

! **Caution:** Acrylamide 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.

There are two different size options for gels using the FMBIO® fluorescence imaging systems, either 32cm × 19cm × 0.4mm (height × width × thickness) or 43cm × 19cm × 0.4mm. The 43cm × 19cm × 0.4mm low-fluorescence glass plates are strongly recommended for better separation. The use of the longer glass plates enables the instrument software to distinguish one-base-pair differences and differences in alleles over 300bp more easily. Also, use of a square-tooth comb provides better separation between lanes.

1. Etch each glass plate on one side in one corner with a diamond tip pencil to distinguish the gel sides of the glass plates. Thoroughly clean the glass plates twice with 95% ethanol and Kimwipes® tissues.

Note: If using a square-tooth comb, the shorter glass plate requires bind silane treatment (see below). The plates do not require a special silane treatment when using a sharktooth comb.



5.A. Polyacrylamide Gel Preparation (continued)

Bind Silane Treatment of Glass Plate

Prepare fresh binding solution in a chemical fume hood. Add 1.5µl of bind silane to a 1.5ml microcentrifuge tube containing 0.5ml of 0.5% acetic acid in 95% ethanol. Wipe the etched side of the shorter glass plate in the comb region using a Kimwipes® tissue saturated with the freshly prepared binding solution. Wait 5 minutes for the binding solution to dry. Wipe the shorter glass plate 3–4 times with 95% ethanol and Kimwipes® tissues in the comb area to remove the excess binding solution.

2. Assemble the glass plates by placing 0.4mm side spacers between the plates using 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.
3. Prepare a 4% or 6% acrylamide solution by combining the ingredients listed in Table 4.

Note: If preparing multiple gels on a daily basis, a larger 4% or 6% stock solution may be prepared, filtered as in Step 4 below, and stored at 4°C in the dark for up to one month. To prepare a single gel, remove 30ml of this stock solution and continue with Step 5.

Table 4. Preparation of 4% and 6% Polyacrylamide Gels.

Component	4% Gel (32cm)	4% Gel (43cm)	6% Gel (32cm)	6% Gel (43cm)	Final Concentration
urea	12.6g	18.9g	12.6g	18.9g	7M
deionized water	16.0ml	24.0ml	14.5ml	21.75ml	-
10X TBE	1.5ml	2.25ml	1.5ml	2.25ml	0.5X
40% acrylamide:bis	3.0ml	4.5ml	4.5ml	6.75ml	4% or 6%
total volume	30ml	45ml	30ml	45ml	

4. Filter the acrylamide solution through a 0.2 micron filter (e.g., Nalgene® tissue culture filter).
5. Pour the filtered acrylamide solution into a squeeze bottle.
6. Add the appropriate amounts of TEMED and 10% ammonium persulfate to the acrylamide solution and mix gently.

Component	32cm Gel (30ml)	43cm Gel (45ml)
TEMED	20µl	30µl
10% ammonium persulfate	200µl	300µl

7. 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 the movement of the solution to the bottom of the plates while maintaining a constant flow of the solution. When the solution begins to flow out from the bottom, position the plates horizontally.

8. Insert the square-tooth comb between the glass plates until the teeth are almost completely inserted into the gel, **or** insert one 14cm doublefine (49 point) sharktooth comb, straight side into the gel, between the glass plates (6mm of the comb should be between the two glass plates).
9. Secure the comb with 3 evenly spaced clamps.
10. Pour the remaining acrylamide solution into a disposable conical tube as a polymerization control. Rinse the squeeze bottle, including the spout, with water.
11. 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 of the gel 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 paper towels saturated with deionized water.
2. Shave any excess polyacrylamide away from the comb, and remove the comb.
3. Add TBE 0.5X 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 TBE 0.5X buffer to the top buffer 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 40–45 watts for 30 minutes for a 32cm gel or 60–65 watts for 30 minutes for a 43cm gel. The gel running conditions may need to be adjusted to reach a temperature of 50°C.
9. Prepare the sample and allelic ladder samples during the gel pre-run.



5.C. Sample Preparation and Loading

The Fluorescent Ladder (CXR), 60–400 Bases, is included in the PowerPlex® 1.1 System for those who wish to use an internal size marker for three-color detection and analysis of amplified samples. With this approach, only 2–3 lanes of the PowerPlex® 1.1 Allelic Ladder are required per gel. Alternatively, the two-color detection method may be employed in which the Fluorescent Ladder (CXR) is not used and the PowerPlex® 1.1 Allelic Ladder Mix is loaded as often as every third gel lane.

1. Prepare the amplified samples and ladders as described below, depending on the detection method employed (either three-color detection or two-color detection).

For three-color detection (using the Fluorescent Ladder (CXR), 60–400 Bases)

- a. Prepare a loading cocktail by combining and mixing the Fluorescent Ladder (CXR) and Bromophenol Blue Loading Solution as follows:

$[(1\mu\text{l Fluorescent Ladder}) \times (\# \text{ lanes})] + [(3\mu\text{l Bromophenol Blue Loading Solution}) \times (\# \text{ lanes})]$

- b. Combine 4 μl of the prepared loading cocktail and 2 μl of amplified sample.

Note: If the fluorescent signal on the gel is too intense, either dilute the samples in STR 1X Buffer before mixing with loading cocktail or use less DNA template in the amplification reactions.

- c. Combine 4 μl of the prepared loading cocktail and 2 μl of the PowerPlex® 1.1 Allelic Ladder Mix.

Notes:

To analyze the **PowerPlex® 1.1 System with Amelogenin** reactions, mix the corresponding allelic ladders 3:1 (i.e., 3 parts PowerPlex® 1.1 Allelic Ladder with 1 part Amelogenin Allelic Ladder) before mixing with loading cocktail. The number of ladder lanes depends on personal preference and the number of samples analyzed.

To use the **TH01 Allele 9.3** alone, mix 0.5 μl of allele 9.3 with 1.5 μl of 1X STR Buffer and 4 μl of loading cocktail. To use the TH01 Allele 9.3 in combination with the PowerPlex® 1.1 Allelic Ladder, mix 0.5 μl of allele 9.3 with 1.5 μl of PowerPlex® 1.1 Allelic Ladder and 4 μl of loading cocktail.

Do not use fluorescein-labeled TH01 Allele 9.3.

For two-color detection (not using the Fluorescent Ladder (CXR), 60–400 Bases)

- a. Combine 2.5µl of Bromophenol Blue Loading Solution and 2.5µl of amplified sample.

Note: If the fluorescent signal is too intense, either dilute the samples in STR 1X Buffer before mixing with loading solution or use less DNA template in the amplification reactions.

- b. Combine 2.5µl of Bromophenol Blue Loading Solution and 2.5µl of the PowerPlex® 1.1 Allelic Ladder Mix.

Notes:

To analyze the **PowerPlex® 1.1 System with Amelogenin** reaction, mix the corresponding allelic ladders 3:1 (i.e., 3 parts PowerPlex® 1.1 Allelic Ladder with 1 part Amelogenin Allelic Ladder) before mixing with Bromophenol Blue Loading Solution. The number of ladder lanes depends on personal preference and the number of samples analyzed.

To use the **TH01 Allele 9.3** alone, mix 0.5µl of allele 9.3 with 2µl of 1X STR Buffer and 2.5µl of Bromophenol Blue Loading Solution. To use the TH01 Allele 9.3 in combination with the PowerPlex® 1.1 Allelic Ladder, mix 0.5µl of allele 9.3 with 2µl of PowerPlex® 1.1 Allelic Ladder and 2.5µl of Bromophenol Blue Loading Solution.

2. (Optional). Place 6µl of Gel Tracking Dye 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 3 lanes from the nearest sample and is used as a visual indicator of migration.
3. Briefly centrifuge the samples in a microcentrifuge to bring the contents to the bottom of the tubes.
4. Denature the samples by heating at 95°C for 2 minutes, and immediately chill on crushed ice or in an ice-water bath. Denature the samples **just** prior to loading the gel.
5. After the pre-run use a 50–100cc syringe filled with buffer to flush the urea from the well area. If using a square-tooth comb, do not reinsert the comb, as the samples will be loaded directly into the wells. If using a sharktooth comb, insert the teeth into the gel approximately 1–2mm, and leave the comb inserted in the gel during both gel loading and electrophoresis.
6. 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 (Gel Pre-Run). Observe the lane containing the Gel Tracking Dye to monitor the migration of the samples.

Note: In a 6% denaturing polyacrylamide gel, the bromophenol blue component of the Gel Tracking Dye migrates at approximately 25 bases, and the xylene cyanol component migrates at approximately 105 bases.

In a 4% denaturing polyacrylamide gel, the bromophenol blue component migrates at approximately 40 bases, and the xylene cyanol component migrates at approximately 170 bases.

2. Based on the size ranges for each locus (Section 9.B, Table 7) and the migration characteristics of the dyes contained in the Gel Tracking Dye, stop electrophoresis before the smallest locus (i.e., D5S818) has reached the bottom of the gel.

5.E. Detection

1. After electrophoresis, remove the gel/glass plate unit from the apparatus. Remove the comb and side spacers, but **do not** separate the glass plates.
2. Clean both sides of the gel/glass plate unit with deionized water and paper towels. The plates must be very clean for scanning.
3. Scan the gel according to the parameters listed in Table 5 for each instrument. Use the 505nm filter to detect fluorescein-labeled fragments, the 585nm filter to detect TMR-labeled fragments and the 650nm filter to detect the Fluorescent Ladder (CXR), 60–400 Bases. Different laboratories may wish to modify these parameters according to their personal preferences.

Table 5. Instrument Parameters for the Hitachi FMBIO® and FMBIO® II Fluorescence Imaging Systems and the PowerPlex® 1.1 System.

	Hitachi FMBIO® Fluorescent Scanner	Hitachi FMBIO® II Fluorescent Scanner
Material Type	acrylamide gel	acrylamide gel
Resolution:		
Horizontal	150dpi	150dpi
Vertical	150dpi	150dpi
Rate	0.1024s/line	NA
Repeat	1 time	256 times
Gray Level Correction Type	range	range
Cutoff Threshold:		
Low (background)	50%	50%
High (signal)	1%	1%
Reading Sensitivity	80%	100% (505nm channel) 80% (585nm channel) 100% (650nm channel)
Focusing Point	NA	0.0mm

NA = not applicable.

5.F. Reuse of Glass Plates

Separate the glass plates, and discard the gel. Clean the glass plates with deionized water and a 1% solution of Liqui-Nox® detergent. The use of Liqui-Nox® detergent is extremely important, as **other kinds of soap can build up on the glass plates**. This will result in low signal and high background on the gels. If the glass plates have a soap residue buildup on them, we recommend soaking in 10% sodium hydroxide for 1 hour and rinsing well in deionized water.

If bind silane is used to fix the gel to the smaller glass plate, soak the plate in 10% sodium hydroxide for 1 hour (or until the gel comes off the plate), and clean as described.

6. Data Analysis

View and analyze the gel image to determine allele designations as recommended in the FMBIO® user's manual. Perform the multicolor separation for all gels containing material amplified using the PowerPlex® 1.1 System. Display the gel image using green for the fluorescein-labeled loci (D16S539, D7S820, D13S317, D5S818 loci), red for the TMR-labeled loci (CSF1PO, TPOX, TH01, vWA loci) and cyan for the Fluorescent Ladder (CXR), 60–400 Bases.

6.A. Controls

Observe the lanes containing the negative controls. They should be devoid of amplification products.

Observe the lanes containing the K562 DNA positive controls. Compare the K562 DNA allelic repeat sizes with the locus-specific allelic ladder. The expected K562 DNA allele sizes for each locus are listed in Section 9.B, Table 8. Figure 1 shows an example of results obtained after amplification of the control K562 DNA using the PowerPlex® 1.1 System. The K562 DNA contains imbalanced alleles at several loci. This result is due to the unusual chromosome content of this cell line used to prepare the DNA and is not a function of the PowerPlex® 1.1 System performance.

6.B. Allelic Ladders

In general, the allelic ladders contain fragments of the same lengths as either several or all known alleles for the locus. The allelic ladder sizes and repeat units are listed in Section 9.B, Table 7. Visual comparison between the 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 size standard, the calculated lengths of the allelic ladder components will differ from those listed in Table 7. This is due to differences in migration resulting from sequence differences between the allelic ladder fragments and those of the internal size standard.

6.C. Results

Figures 2 and 3 show typical results achieved using the PowerPlex® 1.1 System in the three-color detection format as described in Section 5.C. Figure 2, Panel A, displays a 505nm scan of the fluorescein-labeled loci, D16S539, D7S820, D13S317 and D5S818. Figure 2, Panel B, displays a 585nm scan of the TMR-labeled loci, CSF1PO, TPOX, TH01 and vWA. Figure 3 shows a 650nm scan and lane trace of the Fluorescent Ladder (CXR), 60–400 Bases.

Figure 4 shows an example of co-amplification using the PowerPlex® 1.1 System and the *GenePrint*® Fluorescent Sex Identification System – Amelogenin (TMR).

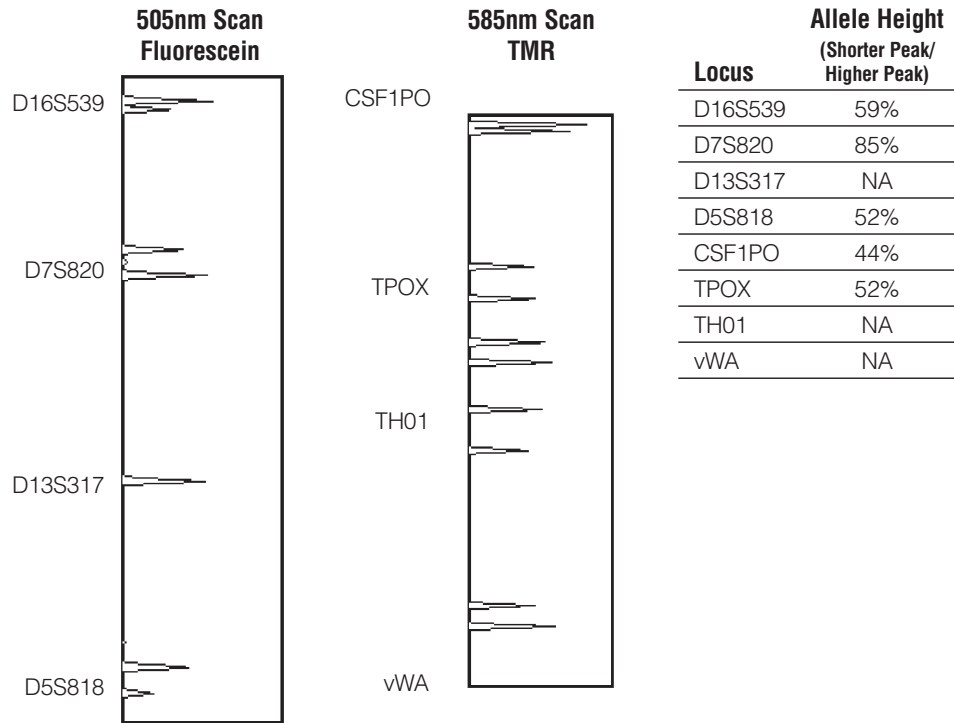


Figure 1. K562 DNA amplified using the PowerPlex® 1.1 System. The fluorescein-labeled loci of the PowerPlex® 1.1 System (D16S539, D7S820, D13S317, D5S818) are shown in the 505nm scan lane trace on the left. The TMR-labeled loci of the PowerPlex® 1.1 System (CSF1PO, TPOX, TH01, vWA) are shown in the 585nm scan lane trace on the right. All eight loci were amplified in one reaction and analyzed in a single gel lane. The table to the right indicates the percentage differences in allele height (i.e., peak heights minus typical inter-locus background value) occurring as a result of the unusual chromosome content of the K562 cell line. These variations are not a consequence of primer imbalance in amplification (see Figures 2 and 4).

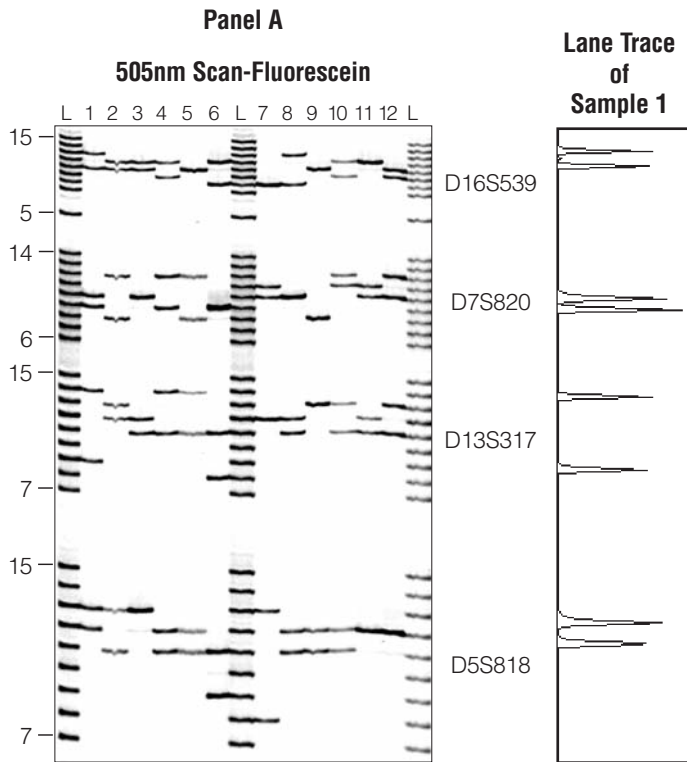


Figure 2. The PowerPlex® 1.1 System. Twelve DNA samples (lanes 1-12) were amplified and are shown with allelic ladders (lanes L) for each of the eight loci contained in the PowerPlex® 1.1 System. **Panel A.** A scan using a 505nm filter, which reveals a black and white image of the fluorescein-labeled loci, D16S539, D7S820, D13S317 and D5S818. **Panel B.** See next page.

In Panels A and B, each allelic ladder is labeled to the left with the number of copies of the repeated sequence contained within its corresponding largest and smallest alleles. All materials were separated using a 4% denaturing polyacrylamide gel and detected using the Hitachi FMBIO® fluorescent scanner. A lane trace corresponding to the sample shown in lane 1 is provided for each panel.

6.C. Results (continued)

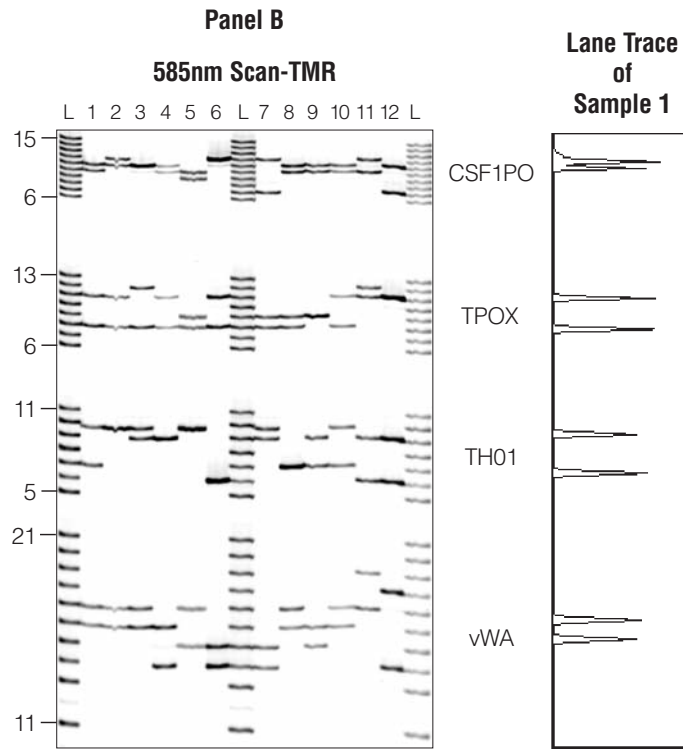


Figure 2. The PowerPlex® 1.1 System. Twelve DNA samples (lanes 1-12) were amplified and are shown with allelic ladders (lanes L) for each of the eight loci contained in the PowerPlex® 1.1 System. **Panel A.** See previous page. **Panel B.** A scan using a 585nm filter, which reveals a black and white image of the TMR-labeled loci, CSF1PO, TPOX, TH01 and vWA.

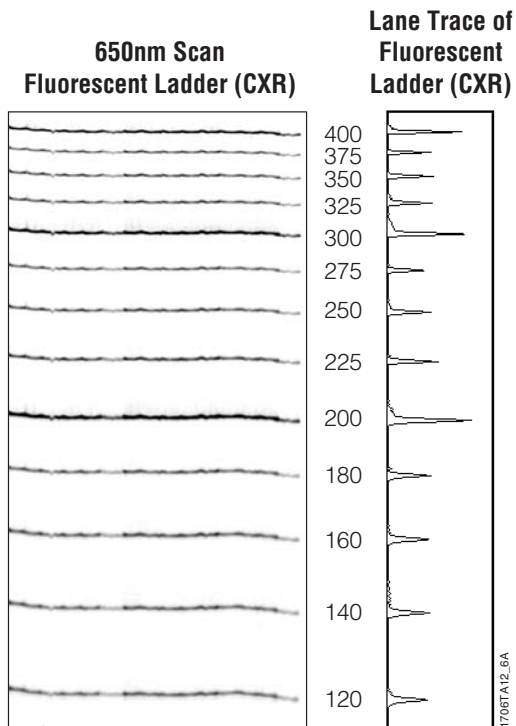


Figure 3. The Fluorescent Ladder (CXR), 60-400 Bases. The Fluorescent Ladder (CXR) was mixed with the samples shown in Figure 2 before gel loading. Following separation, the Fluorescent Ladder (CXR) was detected using a 650nm filter with the Hitachi FMBIO® fluorescent scanner. Fragments smaller than 120 bases are not shown on this gel. Fragment sizes are shown in the middle, and a lane trace is provided. The 100-, 200-, 300- and 400-base fragments display double the intensity of the others.

6.C. Results (continued)

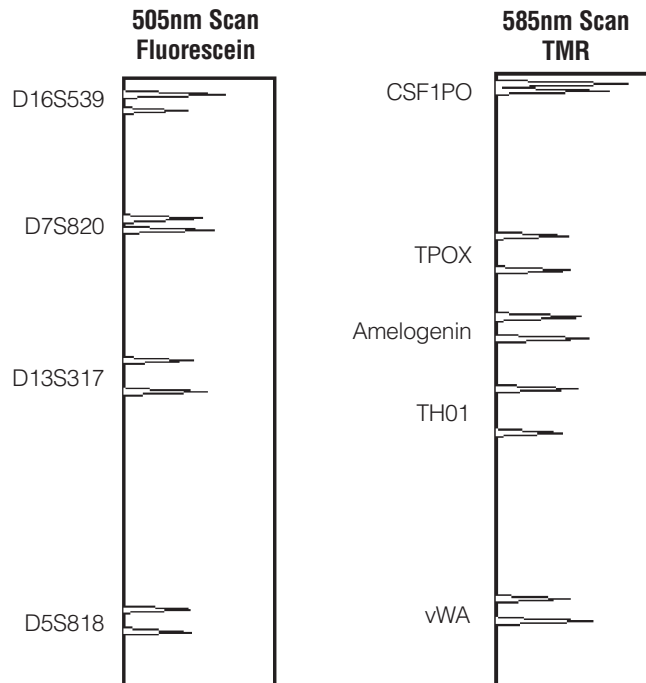


Figure 4. The PowerPlex® 1.1 System and the GenePrint® Fluorescent Sex Identification System – Amelogenin (TMR). The fluorescein-labeled loci of the PowerPlex® 1.1 System (D16S539, D7S820, D13S317, D5S818) are shown in the 505nm scan lane trace on the left. The TMR-labeled loci of the PowerPlex® 1.1 System (CSF1PO, TPOX, TH01, vWA) and Amelogenin are shown in the 585nm scan lane trace on the right. All nine loci were amplified in one reaction and analyzed in a single gel lane.

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 bands	Impure template DNA. Because of the small amount of template used, this is rarely a problem. Depending on the DNA extraction procedure used, inhibitors may be present in the DNA sample.
	Insufficient template DNA. Use the recommended amount of template DNA.
	Insufficient enzyme activity. Use the recommended amount of <i>Taq</i> 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 may also affect PCR. Store DNA in TE ⁻⁴ buffer (10mM Tris HCl [pH 8.0], 0.1mM EDTA) or nuclease-free water.
	Thermal cycler, plate or tube problems. Review the thermal cycling protocols in Section 4.C. We have not tested other reaction tubes, plates or thermal cyclers. Calibrate the thermal cycler heating block if necessary.
	Primer concentration was too low. Use the recommended primer concentration. Mix the PowerPlex® 1.1 Primer Pair Mix well before use.
	Ice was not used to set up reactions. Set up reactions on crushed ice or in an ice-water bath. Very light allele intensity is obtained with some loci if crushed ice or an ice-water bath is not used when setting up reactions. The use of AmpliTaq Gold® DNA polymerase will also remedy this problem.
Bands are fuzzy throughout the lanes	Samples were not denatured before loading. Make sure the samples are heated at 95°C for 2 minutes immediately prior to loading.
	Poor-quality polyacrylamide gel. Prepare acrylamide and buffer solutions using high-quality reagents. Store acrylamide solutions in the dark.
Extra bands visible in one or all of the lanes	Electrophoresis temperature was too high. Run gel at lower temperature (40–60°C).
	Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant tips, and change gloves regularly.
	Artifacts of STR amplification. PCR amplification of STR systems sometimes generates artifacts that appear as faint bands one or four bases below an allele. Refer to Section 9.B for locus-specific information regarding this event.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
Extra bands visible in one or all of the lanes (continued)	Samples were not completely denatured. Heat denature the samples at 95°C for 2 minutes immediately prior to loading.
High background with low signal	Part of the spacers were scanned. Rescan the gel, being careful not to scan any portion of the spacers. Plates were improperly washed. Improper washing of the plates can cause a soap residue to build up on the plates. This can cause background fluorescence.

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9. Appendix

9.A. Advantages of STR Typing

STR typing is more tolerant of the use of degraded DNA templates than other typing methods because the amplification products are less than 500bp long, much smaller than the material detected using AMP-FLP (11) or VNTR (12) analysis. STR typing is also amenable to a variety of rapid DNA purification techniques that are compatible with PCR but do not provide enough DNA of appropriate quality for Southern blot-based analyses.

Amplification products generated with Promega STR products are generally of discrete and separable lengths. This allows the construction of allelic ladders containing fragments of the same lengths as several or all known alleles for each locus. Visual or software-based comparison between the allelic ladder and amplified samples of the same locus allows rapid and precise assignment of alleles. Results obtained using the PowerPlex® 1.1 System can be recorded in a digitized format, allowing direct comparison with stored databases. Population analyses do not require the use of arbitrarily defined fixed bins for population data (13).

9.B. Advantages of Using the Loci Included in the PowerPlex® 1.1 System

The loci included in the PowerPlex® 1.1 System (Table 6) have been selected because they have a high degree of heterozygosity and display a minimum of artifacts. Each STR locus also displays alleles within a limited size range (Table 7). This characteristic allows simultaneous detection of several loci without overlap of alleles across loci. The Sex Identification System – Amelogenin (TMR) is not included in the PowerPlex® 1.1 System but may be simultaneously amplified with this system (see Section 4.B). Table 8 lists the PowerPlex® 1.1 System allele determinations for commonly available standard DNA templates. The PowerPlex® 1.1 System includes K562 DNA as a positive amplification control.

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 (14,15), 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 in sample material or both. The amount of this artifact observed depends primarily on the locus and the DNA sequence being amplified. We have chosen loci that exhibit little or no repeat slippage when detected on the Hitachi FMBIO® platforms. The vWA locus is an exception, revealing as much as 10% stutter. This locus has been included primarily for its popularity with the forensic DNA testing community.

Terminal nucleotide addition (16,17) occurs when *Taq* DNA polymerase adds a nucleotide, generally adenine, to the 3' ends of amplified DNA fragments in a template-independent manner. The efficiency with which this occurs varies with different primer sequences. Thus, an artifact band one base shorter than

expected (i.e., missing the terminal addition) is sometimes seen. We have chosen primer sequences and added a final extension step at 60°C for 30 minutes (18) to the amplification protocol to provide conditions for essentially complete terminal nucleotide addition when the recommended amounts of template DNA are used.

The presence of microvariant alleles (alleles differing from one another by lengths other than the repeat length) complicates separation, 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 (19,20). Therefore, we have selected loci with moderately high polymorphism and minimal occurrence of artifacts.

Table 6. The PowerPlex® 1.1 System and Amelogenin Locus-Specific Information.

STR Locus	Fluorescent Label	Chromosomal Location	GenBank® Locus and Locus Definition	Repeat Sequence ² 5'→3'
Amelogenin ¹	TMR	Xp22.1-22.3 and Y	HUMAMEL, human Y chromosomal gene for Amelogenin-like protein	NA
D16S539	Fluorescein	16q24-qter	NA	GATA
D7S820	Fluorescein	7q11.21-22	NA	GATA
D13S317	Fluorescein	13q22-q31	NA	TATC
D5S818	Fluorescein	5q23.3-32	NA	AGAT
CSF1PO	TMR	5q33.3-34	HUMCSF1PO, human c-fms proto-oncogene for CSF-1 receptor gene	AGAT
TPOX	TMR	2p24-2pter	HUMTPOX, human thyroid peroxidase gene	AATG
TH01	TMR	11p15.5	HUMTH01, human tyrosine hydroxylase gene	AATG (21)
vWA	TMR	12p12-pter	HUMVWFA31, human von Willebrand factor gene	TCTA Complex (21)

¹Amelogenin is not an STR but displays a 212-base, X-specific band and a 218-base, Y-specific band. K562 DNA (female) displays only the 212-base, X-specific band.

²The August 1997 report (22,23) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, "1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5' nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used".

TMR = carboxy-tetramethylrhodamine.

NA = not applicable.

9.B. Advantages of Using the Loci Included in the PowerPlex® 1.1 System (continued)

Table 7. The PowerPlex® 1.1 System and Amelogenin Allelic Ladder Information.

STR Locus	Fluorescent Label	Size Range of Allelic Ladder Components ^{2,3,4} (bases)	Repeat Numbers of Allelic Ladder Components ²	Repeat Numbers of Alleles (Frequency >0.001) Not Present in Allelic Ladder
Amelogenin ¹	TMR	212(X), 218(Y)	NA	None
D16S539	Fluorescein	264-304	5,8-15	None
D7S820	Fluorescein	215-247	6-14	None
D13S317	Fluorescein	165-197	7-15	None
D5S818	Fluorescein	119-151	7-15	None
CSF1PO	TMR	291-327	6-15 ³	None
TPOX	TMR	224-252	6-13 ³	None
TH01	TMR	179-203	5-11	9,3
vWA	TMR	127-167	11,13-21 ³	None

¹Amelogenin is not an STR but displays a 212-base, X-specific band and a 218-base, Y-specific band. K562 DNA (female) displays only the 212 base, X-specific band.

²Lengths of each allele in the allelic ladders have been confirmed by sequence analyses.

³Lengths described in this table may differ from previous versions of allelic ladders for the same loci either because primers have been modified and/or new alleles have been identified (e.g., allelic ladders included with the *GenePrint*® CTTv Fluorescent STR Multiplex do not contain CSF1PO allele 6 or vWA alleles 11 and 21; and allelic ladders included with the *GenePrint*® CTTv Fluorescent STR Multiplex do not contain CSF1PO allele 6, TPOX alleles 6, 7 and 13, and vWA alleles 11 and 21).

⁴When using an internal size standard (e.g., the Fluorescent Ladder (CXR), 60-400 Bases), calculated sizes of allelic ladder components may differ from those listed.

Table 8. The PowerPlex® 1.1 System Allele Determinations in Commonly Available Standard DNA Templates.

STR Locus	Standard DNA Templates ¹		
	K562	9947A	9948 ²
D16S539	12,11	12,11	11,11
D7S820	11,9	11,10	11,11
D13S317	8,8	11,11	11,11
D5S818	12,11	11,11	13,11
CSF1PO	10,9	12,10	12,11,10
TPOX	9,8	8,8	9,8
TH01	9,3,9,3	9,3,8	9,3,6
vWA	16,16	18,17	17,17

¹Strains 9947A and 9948 are available from the NIGMS Human Genetic Mutant Cell Repository (Cornell Institute, Camden, NJ). Strain K562 is available from the American Type Culture Collection (Manassas, VA).

²Strain 9948 displays three alleles at the CSF1PO locus. The band intensity for allele 12 is much lower than that for alleles 10 and 11.

9.C. Additional *GenePrint*[®] Fluorescent STR Products

Table 9 lists the fluorescent STR multiplex systems available from Promega. Three quadriplexes (the GammaSTR[®], CTTv and FFFL Multiplexes) have been developed with fluorescein labels. Use of the PowerPlex[®] 1.1 System and the FFFL System provides analysis of 12 STR loci in two amplification reactions.

Each of the *GenePrint*[®] Fluorescent STR Systems contains all the materials required to perform amplification reactions except for *Taq* DNA polymerase and sample DNA. The corresponding allelic ladders are included in all systems. The Fluorescent Ladder (CXR), 60–400 Bases, is included in the PowerPlex[®] 1.1 System. Additional allelic ladder for any multiplex system or Fluorescent Ladder (CXR), 60–400 Bases, may be purchased separately.

Table 9. *GenePrint*[®] Fluorescent STR Multiplex Systems and PowerPlex[®] 1.1 System.

STR Multiplex System	FL- and TMR-Labeled Loci
CTTv	FL-CSF1PO, FL-TPOX, FL-TH01, FL-vWA
FFFL	FL-F13A01, FL-FESFPS, FL-F13B, FL-LPL
GammaSTR [®]	FL-D16S539, FL-D7S820, FL-D13S317, FL-D5S818
PowerPlex [®] 1.1	FL-D16S539, FL-D7S820, FL-D13S317, FL-D5S818TMR-CSF1PO, TMR-TPOX, TMR-TH01, TMR-vWA

FL = 5'-terminal fluorescein label.

TMR = 5'-terminal carboxy-tetramethylrhodamine label.

9.D. Power of Discrimination

The eight loci in the PowerPlex[®] 1.1 System provide powerful discrimination. Preliminary development of population statistics for these loci, and their various multiplex combinations, has been completed. Generation of these data included analysis of more than two hundred individuals from each of the three major racial and ethnic groups in the United States. These data have been used to generate the commonly employed population statistics displayed in Tables 10–12. For additional population data for STR loci, see references 24–29. Alleles for each system are inherited in Mendelian fashion.

Note: The development of population statistics for these loci has been performed by Promega scientists in collaboration with Robert A. Bever and Steven D. Creacy (Genetic Design, Inc., Greensboro, NC, reference 29).

Table 10 shows the matching probability (30) for the PowerPlex[®] 1.1 System in various populations. The matching probability of the PowerPlex[®] 1.1 System ranges from 1 in 114,000,000 for Caucasian-Americans to 1 in 274,000,000 for African-Americans. The matching probability of the PowerPlex[®] 1.1 System in combination with the *GenePrint*[®] Fluorescent STR Multiplex – F13A01, F13B, FESFPS, LPL (FFFL Multiplex) is 1 in 303,000,000,000 for Caucasian-Americans and 1 in 4,610,000,000,000 for African-Americans.



9.D. Power of Discrimination (continued)

Table 10. Matching Probabilities of the PowerPlex® 1.1 System in Various Populations.

STR System	Matching Probability		
	African-American	Caucasian-American	Hispanic-American
PowerPlex® 1.1 System (8 STR loci)	1 in 2.74×10^8	1 in 1.14×10^8	1 in 1.45×10^8
PowerPlex® 1.1 System with FFFL Multiplex (12 STR loci)	1 in 4.61×10^{12}	1 in 3.03×10^{11}	1 in 4.75×10^{11}

Table 11. Typical Paternity Indices of the PowerPlex® 1.1 System in Various Populations.

STR System	Typical Paternity Index		
	African-American	Caucasian-American	Hispanic-American
PowerPlex® 1.1 System (8 STR loci)	498	260	319
PowerPlex® 1.1 System with FFFL Multiplex (12 STR loci)	8,373	3,976	2,627

Table 12. Power of Exclusion of the PowerPlex® 1.1 System in Various Populations.

STR System	Power of Exclusion		
	African-American	Caucasian-American	Hispanic-American
PowerPlex® 1.1 System (8 STR loci)	.9982	.9969	.9973
PowerPlex® 1.1 System with FFFL Multiplex (12 STR loci)	.99990	.99981	.99974

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 (31). The typical PIs for the PowerPlex® 1.1 System and the PowerPlex® 1.1 System in combination with the FFFL Multiplex are shown in Table 11. The PowerPlex® 1.1 System alone provides typical paternity indices exceeding 260 in each group, enough to satisfy routine requirements for paternity determination. When the FFFL Multiplex is also included, the values exceed 2,600 in all groups.

An alternative calculation used in paternity analyses is the power of exclusion (31). The value calculated for the PowerPlex® 1.1 System exceeds 0.9969 in all populations tested (Table 12). In combination with the FFFL Multiplex, the values exceed 0.9997, demonstrating the usefulness of these two systems for paternity analyses as well as for forensic determinations.

9.E. DNA Extraction and Quantitation Methods

The DNA IQ™ System (Cat.# DC6700) is a DNA isolation and quantitation system designed specifically for forensic and paternity samples (32). 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.J for ordering information.

For applications requiring human-specific DNA quantification, the Plexor® HY System (Cat.# DC1000) has been developed (33). See Section 9.J for ordering information.

The DNA IQ™ System has been fully automated on the Beckman Coulter Biomek® 2000 Laboratory Automation Workstation (34), Biomek® 3000 Laboratory Automation Workstation (35) and Tecan Freedom EVO® Liquid Handler (36). In addition, the DNA IQ™ Reference Sample Kit for Maxwell® 16 (Cat.# AS1040) and DNA IQ™ Casework Sample Kit for Maxwell® 16 are available (37,38). For information on automation of laboratory processes on automated workstations, contact your local Promega Branch Office or Distributor (contact information available at: www.promega.com/worldwide/) or e-mail: genetic@promega.com

9.F. The Fluorescent Ladder (CXR), 60–400 Bases

The Fluorescent Ladder (CXR), 60–400 Bases, contains 16 evenly spaced DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375 and 400 bases in length. Each fragment is labeled with carboxy-X-rhodamine (CXR) and can be detected separately (as a third color) in the presence of PowerPlex® 1.1-amplified material using the Hitachi FMBIO® and FMBIO® II fluorescence imaging systems or Applied Biosystems DNA sequencers. This ladder may be used as an internal size standard in each lane to increase precision in analyses when using the PowerPlex® 1.1 System. Inclusion of the Fluorescent Ladder (CXR) in each lane reduces the number of allelic ladder lanes needed per gel and, therefore, increases the number of lanes available for samples. A protocol for preparation and use of this size standard is provided in Section 5.C.

9.G. Preparing the PowerPlex® 1.1 System Master Mix

Worksheets to calculate the required amount of each component of the PCR master mix are provided in Tables 13 and 14. Multiply the volume per reaction by the total number of reactions to obtain the final master mix volume.

Table 13. Master Mix for the PowerPlex® 1.1 System.

PCR Master Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume (μl)
STR 10X Buffer	2.5μl	×		=	
PowerPlex® 1.1 10X					
Primer Pair Mix	2.5μl	×		=	
<i>Taq</i> DNA polymerase ¹	0.4μl (2u)	×		=	
nuclease-free water ²	μl	×		=	
Per tube		×		=	
template DNA volume ² (1–2ng)	up to 19.6μl	×		=	
total reaction volume	25μl	×		=	

Table 14. Master Mix for the PowerPlex® 1.1 System with Co-Amplification of Amelogenin.

PCR Master Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume (μl)
STR 10X Buffer	2.5μl	×		=	
PowerPlex® 1.1 10X					
Primer Pair Mix	2.5μl	×		=	
<i>Taq</i> DNA polymerase ¹	0.45μl (2.25u)	×		=	
nuclease-free water ²	μl	×		=	
Per tube		×		=	
template DNA volume ² (1–2ng)	up to 17.05μl	×		=	
total reaction volume	25μl	×		=	

¹Assumes that the *Taq* DNA polymerase is at 5u/μl. If the enzyme concentration is different, the volume of enzyme used must be adjusted accordingly.

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

9.H. Agarose Gel Electrophoresis of Amplification Products (Optional)

This procedure is optional if PCR is routinely performed in your laboratory. Agarose gel electrophoresis is used to rapidly confirm the success of the amplification reaction prior to performing polyacrylamide gel electrophoresis.

Materials to Be Supplied by the User

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

- TAE 1X buffer
 - agarose
 - 5X loading solution
 - ethidium bromide solution, 0.5 μ g/ml
1. Prepare a 2% agarose gel (approximately 150cm²) by adding 2.0g of agarose to 100ml of TAE 1X buffer. Mark the liquid level on the container, then boil or heat in a microwave oven to dissolve the agarose. Add preheated (60°C) deionized water to make up for any volume lost due to evaporation.
 2. Cool the agarose to 55°C before pouring into the gel tray. Be sure that the gel tray is level. Pour the agarose into the tray, insert the gel comb and allow to set for 20–30 minutes.
 3. Prepare the samples by mixing 10 μ l of each amplified sample with 2.5 μ l of 5X loading solution.
 4. Prepare 1 liter of TAE 1X buffer for the electrophoresis running buffer.
 5. Place the gel and tray in the electrophoresis gel box. Pour enough running buffer into the tank to cover the gel to a depth of at least 0.65cm. Gently remove the comb.
 6. Load each sample mixed with the appropriate volume of 5X loading solution.
 7. Set the voltage at 5 volts/cm (measured as the distance between the two electrodes). Allow the gel to run for 2 hours.
 8. After electrophoresis, stain the gel in TAE 1X buffer containing 0.5 μ g/ml ethidium bromide. Gently rock for 20 minutes at room temperature. Remove the ethidium bromide solution, and replace with deionized water. Allow the gel to destain for 20 minutes.
 9. Photograph the gel using a UV transilluminator (302nm).
- Note:** When analyzing the data, do not be alarmed if you see extra bands in addition to the alleles. DNA heteroduplexes can be expected when performing nondenaturing agarose gel electrophoresis. The sole purpose of the agarose gel is to confirm the success of the PCR amplification.



9.I. Composition of Buffers and Solutions

40% acrylamide:bis (19:1)

Dissolve 380g of acrylamide and 20g of bisacrylamide in 500ml of deionized water. Bring volume to 1 liter with deionized water.

10% ammonium persulfate

Add 0.5g of ammonium persulfate to 5ml of deionized water. Use 200 μ l of 10% ammonium persulfate for each 30ml acrylamide gel solution. Store the remaining volume in 200 μ l aliquots at -20°C.

Bromophenol Blue Loading Solution

10mM	NaOH
95%	formamide
0.05%	bromophenol blue

0.5M EDTA (pH 8.0) stock

186.1g	Na ₂ EDTA • 2H ₂ O
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Add EDTA to 800ml of deionized water with vigorous stirring. Adjust the pH to 8.0 with NaOH (about 20g of NaOH pellets). Adjust final volume to 1 liter. Dispense into aliquots, and sterilize by autoclaving.

ethidium bromide solution (10mg/ml)

1.0g	ethidium bromide
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Dissolve the ethidium bromide in 100ml of deionized water. Wrap it in aluminum foil or transfer the solution to a dark bottle, and store at room temperature.



Caution: Ethidium bromide is a powerful mutagen. Wear gloves when working with the dye, and wear a mask when weighing it.

Gel Tracking Dye

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

5X loading solution

5%	Ficoll® 400
0.1%	bromophenol blue
0.1%	xylene cyanol
100mM	EDTA (Na ₂ EDTA • 2H ₂ O)
10mM	Tris-HCl (pH 7.5)

STR 10X Buffer

500mM	KCl
100mM	Tris-HCl (pH 9.0)
15mM	MgCl ₂
1%	Triton® X-100
2mM	each dNTP

TAE 50X buffer (pH 7.2)

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

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

TBE 10X buffer

107.8g	Tris base
7.44g	EDTA (Na ₂ EDTA • 2H ₂ O)
~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 7.5)]

1.21g	Tris base
0.037g	EDTA (Na ₂ EDTA • 2H ₂ O)

Dissolve Tris base and EDTA in 900ml of deionized water. Adjust to pH 7.5 with HCl. Increase volume to 1 liter with deionized water.

9.J. Related Products

PowerPlex® Systems

Product	Size	Cat.#
PowerPlex® 1.1 and 2.1 Systems	400 reactions	DC6500
	100 reactions	DC6501
PowerPlex® 1.2 System	100 reactions	DC6101
PowerPlex® 2.1 System	400 reactions	DC6470
	100 reactions	DC6471
PowerPlex® 16 System	400 reactions	DC6530
	100 reactions	DC6531
PowerPlex® 16 BIO System	400 reactions	DC6540
	100 reactions	DC6541

Not For Medical Diagnostic Use.

GenePrint® Fluorescent STR Multiplex Systems

Product	Size	Cat.#
GenePrint® Fluorescent STR Multiplex – GammaSTR® (D16S539, D7S820, D13S317, D5S818)	100 reactions	DC6071
	400 reactions	DC6070
GenePrint® Fluorescent STR Multiplex – CSF1PO, TPOX, TH01, vWA (Fluorescein) (CTTv Multiplex)	100 reactions	DC6301
	400 reactions	DC6300
GenePrint® Fluorescent STR Multiplex – F13A01, FESFPS, F13B, LPL (Fluorescein) (FFFL Multiplex)	100 reactions	DC6311
	400 reactions	DC6310

Not For Medical Diagnostic Use.

GenePrint® Fluorescent Sex Identification System

Product	Size	Cat.#
GenePrint® Fluorescent Sex Identification System – Amelogenin (Fluorescein)	100 reactions	DC5171

Not For Medical Diagnostic Use.

Allelic Ladders

Product	Size	Cat.#
Internal Lane Standard 600	150µl	DG1071
CTTv Allelic Ladder Mix (Fluorescein)	150µl	DG2121
FFFL Allelic Ladder Mix (Fluorescein)	150µl	DG2131
GammaSTR® Allelic Ladder Mix (Fluorescein)	150µl	DG3291

9.J. Related Products (continued)

Accessory Components

The following products are qualified for use with the *GenePrint*[®] Fluorescent STR Systems.

Product	Size	Cat.#
Bromophenol Blue Loading Solution	3ml (3 × 1ml)	DV4371
Gel Tracking Dye	1ml (4 × 250µl)	DV4361
Gold ST [*] R 10X Buffer	1.2ml	DM2411
Blue Dextran Loading Solution	3ml (3 × 1ml)	DV4351
STR 2X Loading Solution	3ml (3 × 1ml)	DV4331
STR 10X Buffer	1.2ml	DM2211
Mineral Oil	12ml	DY1151

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 Instrument	each	AS2000
DNA IQ [™] Reference Sample Kit for Maxwell [®] 16**	48 preps	AS1040
DNA IQ [™] Casework Sample Kit for Maxwell [®] 16**	48 preps	AS1210
Plexor [®] HY System*	800 reactions	DC1000
	200 reactions	DC1001
Slicprep [™] 96 Device	10 pack	V1391

*Not for Medical Diagnostic Use.

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

ART[®] Aerosol-Resistant Tips

Product	Volume	Size (tips/pack)	Cat.#
ART [®] 10 Ultramicro Pipet Tip	0.5–10µl	960	DY1051
ART [®] 20E Ultramicro Pipet Tip	0.5–10µl	960	DY1061
ART [®] 20P Pipet Tip	20µl	960	DY1071
ART [®] GEL Gel Loading Pipet Tip	100µl	960	DY1081
ART [®] 100 Pipet Tip	100µl	960	DY1101
ART [®] 100E Pipet Tip	100µl	960	DY1111
ART [®] 200 Pipet Tip	200µl	960	DY1121
ART [®] 1000, Pipet Tip	1,000µl	800	DY1131

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Patents for the foundational PCR process, European Pat. Nos. 201,184 and 200,362, expired on March 28, 2006. In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.

[©]U.S. Pat. Nos. 5,843,660 and 6,221,598, Australian Pat. No. 724531, Canadian Pat. No. 2,118,048, Korean Pat. No. 290332, Singapore Pat. No. 57050, Japanese Pat. No. 3602142 and other patents pending.

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