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Technical Manual

GenePrint® STR Systems (Silver Stain Detection)

INSTRUCTIONS FOR USE OF PRODUCTS DC1191, DC4001, DC4011, DC4021, DC4031, DC4041, DC4051, DC4061, DC4071, DC4080, DC4081, DC6000, DC6001, DC6030, DC6031, DC6450, DC6451, DG2141 AND DG2101.

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GenePrint® STR Systems (Silver Stain Detection)

All technical literature is available on the Internet at: www.promega.com/tbs/
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.
E-mail: geneticv@promega.com.

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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, which often may be detected using PCR (5–8). Alleles of these loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using silver stain or fluorescent detection following electrophoretic separation.

The *GenePrint*® STR Systems[a] provide all of the materials required to perform 100 or 400 amplification reactions except for *Taq* DNA polymerase and sample DNA. Accessory components are available to simplify many of the procedures related to STR analysis (Section 13.I).

This manual describes methods we have evaluated and recommend for sample preparation, amplification of sample, separation of amplified products and silver detection of separated material. All of the *GenePrint*® STR Systems can be amplified using either the Perkin-Elmer model 480 or 9600 thermal cyclers, but slight differences in yield or balance between loci might be observed if the system is not first optimized on that particular thermal cycler.

Information about allele frequencies for African-Americans, Caucasian-Americans and Hispanic-Americans for all currently available STR Systems is available in Section 13.G. Additional population data for STR loci can be found in Edwards *et al*. (3), Puers *et al.* (9), Hammond *et al.* (10), Bever *et al.* (11), Sprecher *et al*. (12) and Lins *et al*. (13).

2. Product Components and Storage Conditions

GenePrint® STR Multiplex Systems (Silver Stain Detection)

Each system contains the appropriate locus-specific primer pair and allelic ladder in addition to STR 10X Buffer, K562 DNA, STR 2X Loading Solution and pGEM® DNA Markers. Multiplex STR Systems include a single tube containing all required 10X primer pairs as a mixture for simultaneous amplification of more than one locus and another tube containing a mixture of the allelic ladders for the same set of loci.

Product	Size	Cat.#
GenePrint® SilverSTR® III System[a,b]	100 reactions	DC6451
	400 reactions	DC6450

Not For Medical Diagnostic Use. Cat.# DC6450 contains sufficient reagents for 400 reactions of 25µl each. Includes:

- 1ml SilverSTR® III 10X Primer Pair Mix
- 600µl SilverSTR® III Allelic Ladder Mix
- 1.2ml STR 10X Buffer
- 3µg K562 DNA High Molecular Weight (10ng/µl)
- 2ml STR 2X Loading Solution
- 3µg pGEM® DNA Markers (20ng/µl)
- 1 Protocol

2. Product Components and Storage Conditions (continued)

Product	Size	Cat.#
GenePrint® STR Multiplex System— CSF1PO, TPOX, TH01 Multiplex(a,c)	100 reactions	DC6001
	400 reactions	DC6000
GenePrint® STR Multiplex System— F13A01, FESFPS, vWA Multiplex(a)	100 reactions	DC6031
	400 reactions	DC6030

Not For Medical Diagnostic Use.

GenePrint® STR Monoplex Systems (Silver Stain Detection)

Each system contains the specific primer pair and ladder plus other components sufficient to perform the specified number of reactions.

Product	Size	Cat.#
GenePrint® Sex Identification System Amelogenin (Silver Detection)	100 reactions	DC4081
GenePrint® STR System—CSF1PO	100 reactions	DC4011
GenePrint® STR System—F13A01	100 reactions	DC4041
GenePrint® STR System—F13B	100 reactions	DC4001
GenePrint® STR System—FESFPS	100 reactions	DC4021
GenePrint® STR System—HPRTB	100 reactions	DC4061
GenePrint® STR System—LPL	100 reactions	DC4071
GenePrint® STR System—TH01	100 reactions	DC1191
GenePrint® STR System—TPOX	100 reactions	DC4051
GenePrint® STR System—vWA	100 reactions	DC4031

Not For Medical Diagnostic Use.

Allelic Ladders(a)

Product	Size	Cat.#
CTT Allelic Ladder Mix	150µl	DG2101
FFv Allelic Ladder Mix	150µl	DG2141

Storage Conditions: Store all components at –20°C. The pre- and post-amplification components (Allelic Ladder Mix, STR 2X Loading Solution and pGEM® DNA Markers) are sealed in separate packages to prevent cross-contamination. We strongly recommend that pre-amplification and post-amplification reagents be stored separately and handled with different pipettes, tube racks, etc. Store amplified material at –20°C.

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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 (14,15). 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 amplification. We suggest strict adherence to recommended procedures for amplification, denaturing gel electrophoresis, silver stain analysis and recording of data on film.

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, setting up amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (STR 10X Buffer, K562 Control DNA and 10X Primer Pairs) are provided in a separate box and should be stored separately from those used following amplification (Allelic Ladders, STR 2X Loading Solution and pGEM® DNA Markers). Always include a negative control reaction (i.e., no template) to detect reagent contamination. We highly recommend the use of gloves and aerosol-resistant pipet tips.

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
acetic acid (fix/stop solution)	corrosive, hygroscopic
acrylamide	suspected carcinogen, toxic
ammonium persulfate	oxidizer, corrosive
bisacrylamide	toxic, irritant
formaldehyde (staining solution and developer solution)	highly toxic, suspected carcinogen
formamide (STR 2X Loading Solution)	irritant, teratogen
methacryloxypropyltrimethoxysilane (bind silane)	toxic, moisture sensitive
silver nitrate (staining solution)	highly toxic, oxidizer
sodium thiosulfate (developer solution)	irritant, hygroscopic
TEMED	corrosive, flammable
urea	irritant
xylene cyanol FF (STR 2X Loading Solution)	irritant

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4. Amplification

The *GenePrint*® STR Systems have 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, use the GoldST*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 modified *Taq* DNA polymerase is optimized at pH 8.3. Also, when using AmpliTaq Gold® DNA polymerase, incorporate an additional incubation at 95°C for 11 minutes prior to initiation of the thermal cycling program.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 13.F.)

- thermal cycler, model 480 or GeneAmp® system 9600 (Perkin-Elmer)
- microcentrifuge
- *Taq* DNA polymerase
- Nuclease-Free Water (Cat.# P1193 or equivalent)
- Mineral Oil (Cat.# DY1151 or equivalent)
- 0.5ml or 0.2ml microcentrifuge tubes (compatible with thermal cycler)
- 1.5ml microcentrifuge tubes
- BSA Fraction V (optional)
- aerosol-resistant pipet tips
- crushed ice

4.A. Choice of Thermal Cycling Protocol

The CTT and FFv multiplexes, their corresponding monoplexes, the *GenePrint*® Sex Identification System—Amelogenin and the *GenePrint*® STR System—HPRTB are optimized for use with Perkin-Elmer GeneAmp® reaction tubes and the Perkin-Elmer model 480 thermal cycler. The SilverSTR® III System is optimized for use with MicroAmp® tubes and the GeneAmp® PCR system 9600 thermal cycler. However, each system may be used with either thermal cycler.

Please refer to Tables 2 and 3 for recommended and alternative protocols for each system and thermal cycler. The cycling conditions for each protocol are given in Table 4. When using a thermal cycler on which a system was not optimized, there may be a small loss in product yield or sensitivity, and the balance between loci may change slightly in the multiplex systems. Meticulous care must be taken to ensure successful amplification. A guide to amplification troubleshooting is provided in Section 11.

Table 2. Protocol Options for the Model 480 Thermal Cycler.

GenePrint® STR System	Recommended Protocols[1]	Alternative Protocols[2]
Multiplexes		
CTT Multiplex	2	1
CTT Multiplex with Amelogenin	2	1
FFv Multiplex	7	1
SilverSTR® III Multiplex[3]	7	NA
Individual Systems		
Amelogenin	2	1
CSF1PO, F13A01, TH01 or TPOX	2	NA
F13B, LPL or vWA	7	1
FESFPS or HPRTB	1	NA

NA = not applicable.

[1]Recommended protocols offer similar performance characteristics.

[2]Alternative protocols also work but may trade off performance characteristics, such as greater speed or convenience, for less sensitivity.

[3]Performance variation of thermal cyclers may cause extraneous bands to be generated above the allele range. See Section 9.

Table 3. Protocol Options for the GeneAmp® PCR System 9600 Thermal Cycler.

GenePrint® STR System	Recommended Protocols[1]	Alternative Protocols[2]
Multiplexes		
CTT Multiplex	5,6	12
CTT Multiplex with Amelogenin	5,6	12
FFv Multiplex	8,9	3,4
SilverSTR® III Multiplex[3]	10	NA
Individual Systems		
Amelogenin, CSF1PO, F13A01, TH01 or TPOX	5,6	NA
F13B, FESFPS, HPRTB, LPL or vWA	3,4	NA

NA = not applicable.

[1]Recommended protocols offer similar performance characteristics.

[2]Alternative protocols also work but may trade off performance characteristics, such as greater speed or convenience, for less sensitivity.

[3]Performance variation of thermal cyclers may cause extraneous bands to be generated above the allele range. See Section 9.

Table 4. Amplification Protocols.

Protocol Number	Thermal Cycler[1]	Initial Incubation[2]	Programmed Ramp Times	Cycling for First 10 Cycles	Programmed Ramp Times	Cycling for Last 20 Cycles	Extension Step	Hold Step
1 (Refer to Note 1)	480	96°C for 2 minutes	None	94°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	None	90°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	None	4°C
2 (Refer to Note 1)	480	96°C for 2 minutes	None	94°C, 1 minute 64°C, 1 minute 70°C, 1.5 minutes	None	90°C, 1 minute 64°C, 1 minute 70°C, 1.5 minutes	None	4°C
3 (Refer to Note 2)	9600	96°C for 2 minutes	None	94°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	None	90°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	None	4°C
4 (Refer to Note 3)	9600	96°C for 2 minutes	50 seconds to 94°C, 1 minute 34 seconds to 60°C, 1 minute 25 seconds to 70°C, 1.5 minutes		45 seconds to 90°C, 1 minute 30 seconds to 60°C, 1 minute 25 seconds to 70°C, 1.5 minutes		None	4°C
5 (Refer to Note 2)	9600	96°C for 2 minutes	None	94°C, 1 minute 64°C, 1 minute 70°C, 1.5 minutes	None	90°C, 1 minute 64°C, 1 minute 70°C, 1.5 minutes	None	4°C
6 (Refer to Note 3)	9600	96°C for 2 minutes	50 seconds to 94°C, 1 minute 30 seconds to 64°C, 1 minute 15 seconds to 70°C, 1.5 minutes		45 seconds to 90°C, 1 minute 26 seconds to 64°C, 1 minute 15 seconds to 70°C, 1.5 minutes		None	4°C
7 (Refer to Note 1)	480	96°C for 2 minutes	None	94°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	None	90°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	60°C for 30 minutes	4°C
8 (Refer to Note 2)	9600	96°C for 2 minutes	None	94°C, 1minute 60°C, 1 minute 70°C, 1.5 minutes	None	90°C, 1 minute 60°C, 1 minute 70°C, 1.5 minutes	60°C for 30 minutes	4°C

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Table 4. Amplification Protocols (continued).

Protocol Number	Thermal Cycler[1]	Initial Incubation[2]	Programmed Ramp Times	Cycling for First 10 Cycles	Programmed Ramp Times	Cycling for Last 20 Cycles	Extension Step	Hold Step
9 (Refer to Note 3)	9600	96°C for 2 minutes	50 seconds to 94°C, 34 seconds to 60°C, 25 seconds to 70°C,	30 seconds 1 minute 1.5 minutes	45 seconds to 90°C, 30 seconds to 60°C, 25 seconds to 70°C,	1 minute 1 minute 1.5 minutes	60°C for 30 minutes	4°C
10 (Refer to Note 4)	9600	96°C for 1 minute	Default ramp to 94°C, 68 seconds to 60°C, 50 seconds to 70°C,	30 seconds 30 seconds 45 seconds	Default ramp to 90°C, 60 seconds to 60°C, 50 seconds to 70°C,	30 seconds 30 seconds 45 seconds	60°C for 30 minutes	4°C
11 (Refer to Note 4)	9600	96°C for 2 minutes	50 seconds to 94°C, 34 seconds to 60°C, 25 seconds to 70°C,	1 minute 1 minute 1.5 minutes	45 seconds to 90°C, 30 seconds to 60°C, 25 seconds to 70°C,	1 minute 1 minute 1.5 minutes	60°C for 30 minutes	4°C
12 (Refer to Note 4)	9600	96°C for 2 minutes	50 seconds to 94°C, 30 seconds to 64°C, 15 seconds to 70°C,	1 minute 1 minute 1.5 minutes	45 seconds to 90°C, 26 seconds to 64°C, 15 seconds to 70°C,	1 minute 1 minute 1.5 minutes	None	4°C

[1]480 refers to the Perkin-Elmer model 480 thermal cycler; 9600 refers to the Perkin-Elmer GeneAmp® PCR system 9600 thermal cycler.

[2]Initial incubation performed using AmpliTaq® DNA polymerase. When using AmpliTaq Gold® DNA polymerase, include an additional incubation at 95°C for 11 minutes prior to initiation of the thermal cycling program. Also when using AmpliTaq® DNA polymerase, be sure to use the GoldST*R 10X Buffer.

Notes for Table 4:

1. Use GeneAmp® reaction tubes, and overlay all reactions with mineral oil.
2. Use GeneAmp® reaction tubes in combination with the GeneAmp® thin-walled tray. This reduces the maximum number of simultaneous reactions to 48 due to the spacing of holes in the tray. Add mineral oil to all reactions.
3. Use MicroAmp® reaction tubes in the MicroAmp® 9600 tray. This allows a maximum of 96 simultaneous reactions. Add mineral oil to all reactions.

Do not cover the reactions with the system 9600 thermal cycler lid. Cover the reaction tubes loosely with aluminum foil.

Optional: Add BSA Fraction V (final concentration 60µg/ml) to all reactions. This may result in a slight increase in yield. It will also produce higher silver background in the gel lanes. We recommend Sigma BSA (Cat.# A2153). Performance may vary depending on the source of this component.

4. Use MicroAmp® reaction tubes in the MicroAmp® 9600 tray. This allows a maximum of 96 simultaneous reactions. No mineral oil is needed.

Cover reactions with the system 9600 thermal cycler lid.

Optional: Add BSA Fraction V (final concentration 60µg/ml) to all reactions. This may result in a slight increase in yield. It will also produce higher silver background in the gel lanes. We recommend Sigma BSA (Cat.# A2153). Performance may vary depending on the source of this component.

4.B. Amplification Setup

The use of gloves and aerosol-resistant pipet tips is highly recommended to prevent cross-contamination. Helpful organizational sheets are provided in Section 13.H.

1. Thaw the STR 10X Buffer and 10X Primer Pair(s), and place on ice.

   Notes:

   1. Mix reagents by vortexing for 15 seconds before each use.
   2. If using AmpliTaq Gold® DNA polymerase, use the GoldST*R 10X Buffer (Cat.# DM2411) instead of the STR 10X Buffer.

2. Place one clean, autoclaved 0.5ml reaction tube for each reaction into a rack, and label appropriately.

   Note: If using the GeneAmp® PCR system 9600 thermal cycler, refer to the notes for Table 4 for tube selection.

3. Determine the number of reactions to be set up. This should include a positive and negative control reaction. 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.

4.B. Amplification Setup (continued)

4. Calculate the required amount of each component of the PCR master mix (Table 5). Multiply the volume (µl) per sample by the total number of reactions (from Step 3) to obtain the final volume (µl).

   Note: The CTT Multiplex and Amelogenin locus can be amplified simultaneously.

5. In the order listed in Table 5, add the final volume of each reagent to a sterile tube. Mix gently (do not vortex), and place on ice.

   Note: The volume given assumes a *Taq* DNA polymerase concentration of 5u/µl. For different enzyme concentrations, the volume of enzyme added must be adjusted accordingly. If the final 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, and add a larger volume. The amount of sterile water should be adjusted accordingly so that the final volume per reaction is 25µl. Do not store diluted *Taq* DNA polymerase.

Table 5. PCR Amplification Reaction Setup.

Multiplex Reactions Containing Three Loci

PCR Master Mix Component	Volume Per Sample (µl)	×	Number of Reactions	=	Final Volume (µl)
sterile water	17.35				
STR 10X Buffer	2.50				
Multiplex 10X Primer Pair Mix	2.50				
Taq DNA polymerase (at 5u/µl)	0.15 (0.75u)				
total volume	22.50				

Combined CTTv Multiplex and Amelogenin Reactions

PCR Master Mix Component	Volume Per Sample (µl)	×	Number of Reactions	=	Final Volume (µl)
sterile water	14.85				
STR 10X Buffer	2.50				
CTT Multiplex 10X Primer Pair Mix	2.50				
Amelogenin 10X Primer Pair	2.50				
Taq DNA polymerase (at 5u/µl)	0.15 (0.75u)				
total volume	22.50				

Monoplex or Amelogenin-Only Reactions

PCR Master Mix Component	Volume Per Sample (µl)	×	Number of Reactions	=	Final Volume (µl)
sterile water	17.45				
STR 10X Buffer	2.50				
locus-specific 10X primer pair	2.50				
Taq DNA polymerase (at 5u/µl)	0.05 (0.25u)				
total volume	22.50				

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6. Add 22.5µl of PCR master mix to each tube, and place on ice.

(!) 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 2.5µl of each sample into the respective tube containing 22.5µl of PCR master mix.

   Notes:

   1. For the multiplex CTT and SilverSTR® III Systems, use 1–5ng of template DNA.

      For the multiplex FFv System, use 5–10ng of template DNA.

   2. If the template DNA is stored in TE buffer (10mM Tris-HCl, 1mM EDTA [pH 7.5]), 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) or available magnesium concentration (due to chelation by EDTA). DNA samples stored or diluted in sterile, deionized water are not subject to this caution.

8. For the positive amplification control, pipet 2.5µl (5ng) of K562 DNA (diluted to 2ng/µl) into a 0.5ml reaction tube containing 22.5µl of PCR master mix.

9. For a negative amplification control, pipet 2.5µl of sterile water (instead of template DNA) into a 0.5ml reaction tube containing 22.5µl of PCR master mix.

10. If recommended by the cycling protocol, add 1 drop of mineral oil to each tube. Close the tubes.

    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 contents to the bottom of the tube.

4.C. Amplification Thermal Cycling

1. Place the tubes in a thermal cycler.

2. Select and run a recommended protocol from Table 2 or 3 (Section 4.A).

3. After completion of the thermal cycling protocol, store the samples at –20°C.

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

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5. Polyacrylamide Gel Preparation

Materials to Be Supplied by the User
(Solution compositions are provided in Section 13.F.)

- 40% acrylamide:bis (19:1) and TEMED
- 10X TBE Buffer (Cat.# V4251)
- 10% Ammonium Persulfate (Cat.# V3131)
- Urea (Cat.# V3171)
- bind silane (methacryloxypropyltrimethoxysilane)
- Gel Slick® solution (Cambrex Cat.# 50640)
- 0.5% acetic acid in 95% ethanol
- Nalgene® tissue culture filter (0.2 micron)
- polyacrylamide gel electrophoresis apparatus for gels ≥30cm (e.g., SA32 or S2)
- glass plates and side spacers for polyacrylamide gel ≥30cm
- 14cm vinyl doublefine sharkstooth comb(s), 49 point, 0.4mm thick; or square-tooth comb, 35cm, 60 wells (cut in half for 30 wells/gel), 0.4mm thick (Owl Scientific Cat.# S2S-60A)
- power supply
- Liqui-Nox® detergent (Use of Liqui-Nox® detergent is extremely important, as other kinds of detergent can build up on the glass plates.)
- clamps (e.g., large office binder clips)
- diamond pencil for marking glass plates

5.A. Notes

1. Use 6% acrylamide for the *GenePrint*® SilverSTR® III System. In a 4% gel, DNA strand separation in the locus D16S539 are such that the top strand of one allele overlaps with the bottom strand of the next larger allele. See Figure 2.
2. Unpolymerized 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 powder or solutions.
3. Bind silane is toxic and should be used in a chemical fume hood.
4. The longer glass plate will be treated with Gel Slick® solution to prevent the gel from sticking, and the shorter glass plate will be treated with bind silane to bind the gel. The two plates must be kept apart at all times to prevent cross-contamination.
5. All cleaning utensils (sponges) for the longer glass plates should be kept separate from those for the shorter glass plates to prevent cross-contamination of the binding solution.
6. The shorter glass plate preparation must be repeated for each gel. The longer glass plate preparation must be repeated after every four gels.

7. To remove the glass plate treatments (Gel Slick® solution or bind silane) immerse the plate(s) in 10% NaOH solution for 1 hour. Thoroughly rinse the plate(s) with deionized water, and clean with a detergent. The same 10% NaOH solution may be used for multiple gels.

8. New glass plates should be soaked in 10% NaOH for 1 hour and then rinsed thoroughly with deionized water before use. New plates also should be etched with a diamond pencil in the corner of one side to distinguish the sides of the plates in contact with the gel.

5.B. Procedure

The following protocol is for the preparation of a denaturing polyacrylamide gel with the dimensions of 31.0cm wide × 38.5cm high × 0.4mm thick (e.g., S2 sequencing gel electrophoresis apparatus, Whatman Cat.# 21105-010). Use one-half of the volumes described here for a gel with the dimensions of 17cm wide × 32cm high × 0.4mm thick (e.g., SA32 sequencing gel apparatus, Whatman Cat.# 31096-019).

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

   Note: The gel side is the etched side of the glass plate.

2. Using gloves, apply 3ml of Gel Slick® solution onto the etched side of the longer glass plate. With a dry paper towel, spread the Gel Slick® solution using a circular motion over the entire surface.

3. Wait 5 minutes for the Gel Slick® solution to dry. Remove the excess Gel Slick® solution with a paper towel saturated with deionized water. Finally, dry the glass plate with Kimwipes® tissue.

4. In a chemical fume hood, prepare fresh binding solution by adding 3µl of bind silane to 1ml of 0.5% acetic acid in 95% ethanol in a 1.5ml tube. Wipe the etched side of the shorter glass plate using a Kimwipes® tissue saturated with the freshly prepared binding solution. Be certain to wipe the entire plate surface with the saturated tissue.

5. Wait 5 minutes for the binding solution to dry. Wipe the shorter glass plate 3–4 times with 95% ethanol and Kimwipes® tissues to remove the excess binding solution.

Failure to wipe excess binding solution from the shorter glass plate will cause the gel to stick to both plates, and the gel will be destroyed upon separation of the glass plates after electrophoresis.

6. Take special care not to allow the treated surfaces to touch each other. Assemble the glass plates by placing 0.4mm side spacers and a 0.4mm bottom spacer (optional) between the plates and using clamps to hold them in place. Lean the assembled plates against a test tube rack or other similar support.

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5.B. Procedure (continued)

7. Prepare a 4% or 6% acrylamide solution (total of 75ml) by combining the ingredients listed below.

Component	4% Gel	6% Gel	Final Concentration
urea	31.50g	31.50g	7M
deionized water	40.00ml	36.25ml	–
10X TBE buffer	3.75ml	3.75ml	0.5X
40% acrylamide:bis (19:1)	7.50ml	11.25ml	4% or 6%
total volume	75ml	75ml	

Note: If preparing multiple gels on a daily basis, a larger 4% or 6% stock solution may be prepared, filtered as in Step 8 below, and stored at 4°C in the dark for up to one month. To prepare a single gel, remove 75ml of this stock solution, and continue with Step 9.

8. Filter the acrylamide solution through a 0.2 micron filter (e.g., Nalgene® tissue culture filter).

9. Pour the filtered acrylamide solution into a squeeze bottle.

10. Add 50µl of TEMED and 500µl of 10% ammonium persulfate to the acrylamide solution, and mix gently.

11. Carefully pour the acrylamide solution between the glass plates. To prevent bubble formation, start pouring at one side of the assembled plates and maintain a constant flow of solution.

12. Position the gel horizontally, resting it on two test tube racks or other similar supports. Remove any bubbles that may have formed.

13. Insert one or two 14cm doublefine (49 point) sharkstooth combs, straight side into the gel, between the glass plates (6mm of the comb should be between the two glass plates). If using a square-tooth comb, insert the comb between the glass plates until the teeth are almost completely inserted into the gel.

14. Secure the comb(s) with 2–3 clamps each.

15. Pour the remaining acrylamide solution into a disposable conical tube as a polymerization control. Rinse the squeeze bottle, including the spout, with water.

16. 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 well end of the gel to prevent the gel from drying out. If no bottom spacer is used, the bottom of the gel should be wrapped.

6. Polyacrylamide Gel Electrophoresis

6.A. Gel Pre-Run

1. Remove the clamps from the polymerized acrylamide gel, and clean the glass plates with paper towels saturated with deionized water.
2. Shave any excess polyacrylamide away from the comb. Remove the comb and bottom spacer.
3. Add 0.5X TBE to the bottom chamber of the electrophoresis apparatus.
4. Gently lower the gel and glass plates into the buffer with the longer plate facing out and the well-side on top.
5. Secure the glass plates to the sequencing gel apparatus.
6. Add 0.5X TBE to the top buffer chamber of the electrophoresis apparatus.
7. Using a 50–100cc syringe filled with buffer, remove the air bubbles on the top of the gel. Be certain the well area is devoid of air bubbles and small pieces of polyacrylamide. Use a syringe with a bent 19-gauge needle to remove the air bubbles between the glass plates on 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 60–65 watts for a 40cm polyacrylamide gel, 40–45 watts for a 32cm gel. The gel running conditions may have to be adjusted in order to reach a temperature of 50°C.

6.B. Sample Preparation

1. Prepare the PCR samples by mixing 2.5µl of each sample with 2.5µl of STR 2X Loading Solution.

   Note: The sample alleles may appear more intense than ladder alleles on the gel, but this should not interfere with allele determination. For more even band intensities, mix 1µl of each sample with 4µl of a premix containing 2.5µl of STR 2X Loading Solution and 1.5µl of STR 1X Buffer.
2. Add 2.5µl (50ng) of pGEM® DNA Markers to 2.5µl of STR 2X Loading Solution for each marker lane.

   Note: We recommend loading pGEM® DNA Markers into the first and last lanes of the gel.
3. Add 2.5µl of the Allelic Ladder Mix to 2.5µl of STR 2X Loading Solution for each allelic ladder lane. The number of allelic ladder lanes used depends on personal preference.

   Note: For combined CSF1PO, TPOX, TH01 multiplex and Amelogenin reactions, mix the corresponding allelic ladder mixes 1:1, then add 2.5µl of allelic ladder mix to 2.5µl of STR 2X Loading Solution.
4. Briefly centrifuge the samples in a microcentrifuge to bring the contents to the bottom of the tube.

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6.C. Sample Loading

1. Denature the samples by heating at 95°C for 2 minutes, then immediately chill on crushed ice or in an ice-water bath.

   Note: Denature the samples just prior to loading the instrument.

2. After the pre-run (Section 6.A), use a 50–100cc syringe filled with buffer to flush the urea from the well area. If using a sharkstooth comb, carefully insert the comb teeth into the gel approximately 1–2mm. Leave the comb inserted in the gel during both gel loading and electrophoresis.

3. Load 3µl of each sample into the respective wells. The loading process should take no longer than 20 minutes to prevent the gel from cooling.

   Note: An organizational sheet for loading a gel is provided in Section 13.H.

6.D. Gel Electrophoresis

1. Once loading is complete, run the gel using the same conditions as in Section 6.A.

   If you are loading the gel multiple times, allow the gel to run 20–30 minutes before loading the next set of samples (Figure 4). This will prevent the samples from overlapping during electrophoresis. (Do not do this with multiplex systems.)

   Note: In a 6% gel, bromophenol blue migrates at approximately 25 bases and xylene cyanol migrates at approximately 105 bases. In a 4% gel, bromophenol blue migrates at approximately 40 bases and xylene cyanol migrates at approximately 170 bases.

2. Knowing the size ranges for each locus (Tables 7 and 8, Section 13.B) and migration characteristics of the dyes (Step 1, above), stop electrophoresis any time after the locus of interest has passed the midpoint of the gel. If running more than one locus or a multiplex, be careful not to run the smallest locus off the bottom of the gel.

3. Proceed to Section 7 for silver stain detection.

7. Silver Staining

This protocol describes the use of the SILVER SEQUENCE™ Staining Reagents (Cat.# Q4132). One system contains sufficient reagents to stain 10 sequencing size gels and includes:

- 500µl Bind Silane
- 20g Silver Nitrate (10 × 2g)
- 60ml Formaldehyde, 37% (20 × 3ml)
- 10ml Sodium Thiosulfate, 10mg/ml (10 × 1ml)
- 600g Sodium Carbonate (10 × 60g)

Materials to Be Supplied by the User
(Solution compositions are provided in Section 13.F.)

- fix/stop solution
- staining solution
- developer solution (chilled to 4–10°C)
- Nalgene® wash tubs (54.1 × 43.5 × 13cm or appropriate size for your system)
- orbital shaker or rocker platform

Use 2 liters of each solution per gel for each step (for a 54.1 × 43.5 × 13cm tray).

7.A. Procedure

1. After electrophoresis, empty the buffer chambers and carefully loosen the gel clamps. Remove the glass plates from the apparatus.
2. Place the gel and glass plates on a flat surface. Remove the comb and side spacers. Use a plastic wedge to carefully separate the two glass plates. The gel should be strongly affixed to the shorter glass plate.
3. Place the gel (attached to the shorter plate) in a shallow plastic tray (e.g., Nalgene® wash tub).
4. To silver stain, follow Steps a–h. Gently agitate during each step.

Steps involving solutions containing formaldehyde should be performed in a chemical hood.

Step	Solution	Time
a.	fix/stop solution (See Note 1)	20 minutes
b.	deionized water	2 minutes
c.	repeat Step b, twice	2 × 2 minutes
d.	staining solution	30 minutes
e.	deionized water (See Note 2)	10 seconds
f.	developer solution	up to 5 minutes (until alleles and ladders are visible)
g.	fix/stop solution (See Note 3)	5 minutes
h.	deionized water	2 minutes

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7.A. Procedure (continued)

Notes:

1. Save the fix/stop solution from Step 4a, to use in Step 4g.
2. The duration of Step 4e is important. The total time from immersion in deionized water to immersion in developer solution should be less than 20 seconds. If the deionized water rinse step does exceed 20 seconds, repeat Step 4d.
3. Add fix/stop solution directly to developer solution to stop developing reaction.

5. Position the gel and shorter plate upright, and allow it to dry overnight.

   For best results, the gel should be completely dried before APC Film development. Alternatively, to create film prints of the gel immediately, cover the gel with plastic wrap, and proceed to Section 8.

   Figure 1, Section 10 shows typical results for the multiplex *GenePrint*® STR Systems. Figure 3, Section 10, shows typical results for the Amelogenin locus and the STR loci, F13B, HPRTB and LPL using a dilution series of 250ng to 0.5ng of template DNA.

7.B. Reuse of Glass Plates

1. For disposal, immerse the plate and affixed gel in a 10% NaOH solution for 1 hour to overnight. Discard the gel, and clean the glass plate with deionized water and a detergent such as Liqui-Nox® detergent. The 10% NaOH solution may be reused for additional gels.
2. All cleaning utensils and sponges for the longer glass plates should be kept separate from those for the shorter glass plates to prevent cross-contamination of the binding solution.

8. Exposure of Film

A direct image may be produced using Automatic Processor Compatible (APC) Film. The image produced on APC Film is the mirror image of the gel. Use of film allows the generation of multiple permanent images with more control over band and background intensity than does development of the gel alone. Handle all plates with gloved hands to avoid fingerprints.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 13.F.)

- white light box
- automatic film processor or film developing tanks
- Automatic Processor Compatible (APC) Film (Cat.# Q4411)

1. In the darkroom with a safelight on, place the dry, stained gel attached to the shorter plate (gel side up) on a white fluorescent light box.

   Note: For best results, the gel should be completely dry before the image is captured with APC film. If capturing an image from a gel that has not been dried, cover the gel with plastic wrap.

2. Position the APC Film, emulsion side down, over the gel to be copied.

   Note: The emulsion side of the film can be identified as the glossy white surface; the nonemulsion side has a gray tint.

3. Place a clean glass plate on top of the film to maintain contact between the gel and film. Turn on the white light box, and expose the film for 1–2 minutes, depending on the gel background level and the intensity of the white light. (This step must be optimized for individual light boxes.)

4. Develop the film as recommended by the manufacturer. APC film may be processed manually or with an automatic film processor. For automatic film processors, follow the manufacturer's instructions.

   Note: The image produced on APC Film is the mirror image of the gel.

5. If there is very little signal, decrease the exposure time used in Step 3. If the film appears brown or black, increase the exposure time.

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9. Data Analysis

For ease of interpretation, allelic ladders should be run in lanes adjacent to each sample. Direct comparison between the allelic ladders and amplified samples of the same locus allows for assignment of alleles. Note that microvariant alleles, such as the TH01 allele 9.3 and the F13A01 allele 3.2, do not co-migrate with allelic ladder fragments. In addition, mutations or rare alleles may be seen occasionally. The migration of such "off-ladder" alleles cannot be predicted.

With silver stain detection, both DNA strands are detected. For some loci, such as TH01, FESFPS and vWA, the difference in the sequence of the opposing strands causes them to migrate at different rates. This results in doublets for each allele (Figure 1). This strand separation may be more pronounced with longer electrophoresis of gels as seen for the sequential loading of TH01 amplification products in Figure 4 (Section 10). Note that in the case of locus F13A01, more pronounced separation of opposing strands is observed with the larger alleles (see Figure 1).

Artifact bands also may be detected with these systems. Shadow banding (16–18) or repeat slippage appears as faint bands one repeat unit (i.e., 4 bases) below the true alleles. This is most pronounced with the vWA locus (Figure 1).

Terminal nucleotide addition occurs when *Taq* DNA polymerase catalyzes nontemplated addition of a nucleotide to the 3´-termini of amplified DNA fragments (18–20). A band that is one base shorter than the expected allele may result from the inefficiency of the terminal nucleotide addition. An artifact band is generated when this terminal addition does not occur with 100% efficiency. This may be visualized as an extra band, as seen in Figure 3 with the LPL and F13B loci. The addition of a final extension step (amplification protocols 7,8,9,10,11; Table 4) increases the amount of product that contains the added terminal nucleotide, thus minimizing the shorter artifact band (18).

For the *GenePrint*® SilverSTR® III System, performance variation of thermal cyclers may cause extraneous bands to be generated above the allele range. The use of AmpliTaq Gold® DNA polymerase with this system may minimize or eliminate these extra bands. Alternatively, raising the annealing temperature to 62°C can also minimize or eliminate these bands. At annealing temperatures higher than 62°C, the amplification of D7S820 alleles may be compromised.

9.A. pGEM® DNA Markers

The pGEM® DNA Markers are visual standards used to confirm allelic size ranges for the loci. The markers consist of fifteen DNA fragments with the following sizes (in base pairs):

2,645	460	126
1,605	396	75
1,198	350	65
676	222	51
517	179	36

9.B Controls

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

Observe the lanes containing the positive K562 DNA positive controls. Compare the K562 DNA allelic repeat sizes with the locus-specific allelic ladder. The expected K562 DNA allele size(s) for each locus are listed in Tables 7 and 8, Section 13.B.

9.C. STR Ladders

Each locus or multiplex has a characteristic allelic ladder. Please refer to Section 13.B for locus-specific allelic ladder information. In general, the allelic ladders contain fragments of the same lengths as either several or all known alleles for the locus. Visual comparison between the allelic ladder and amplified samples of the same locus allows precise assignment of alleles.

10. Representative STR Data

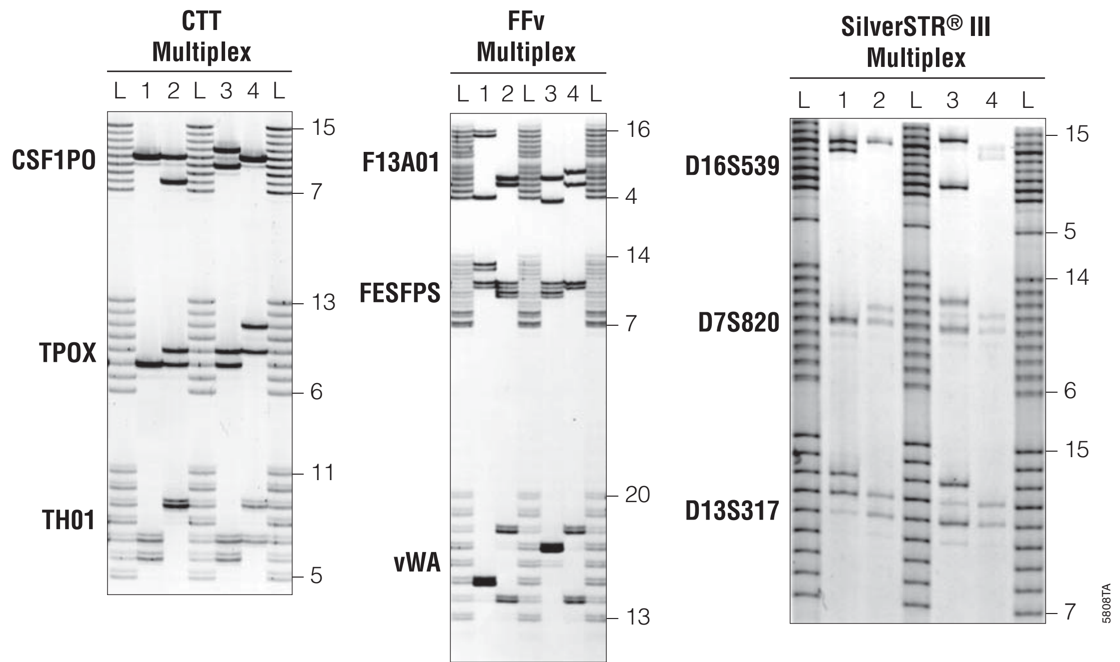

Figure 1. *GenePrint*® STR Multiplex Systems. Individual genomic DNA samples (lanes 1–4) were amplified using *GenePrint*® STR Systems as indicated and detected using silver staining as described in this manual. The amplification products using the CTT (CSF1PO, TPOX, TH01) Multiplex and the FFv (F13A01, FESFPS, vWA) Multiplex were separated using a 4% denaturing polyacrylamide gel. Amplification products using the SilverSTR® III (D16S539, D7S820, D13S317) System were separated in a 6% denaturing polyacrylamide gel. Lanes labeled (L) contain allelic ladders for the respective loci. Numbers to the right of each image indicate the smallest and largest number of repeat units present in corresponding fragments of each allelic ladder.

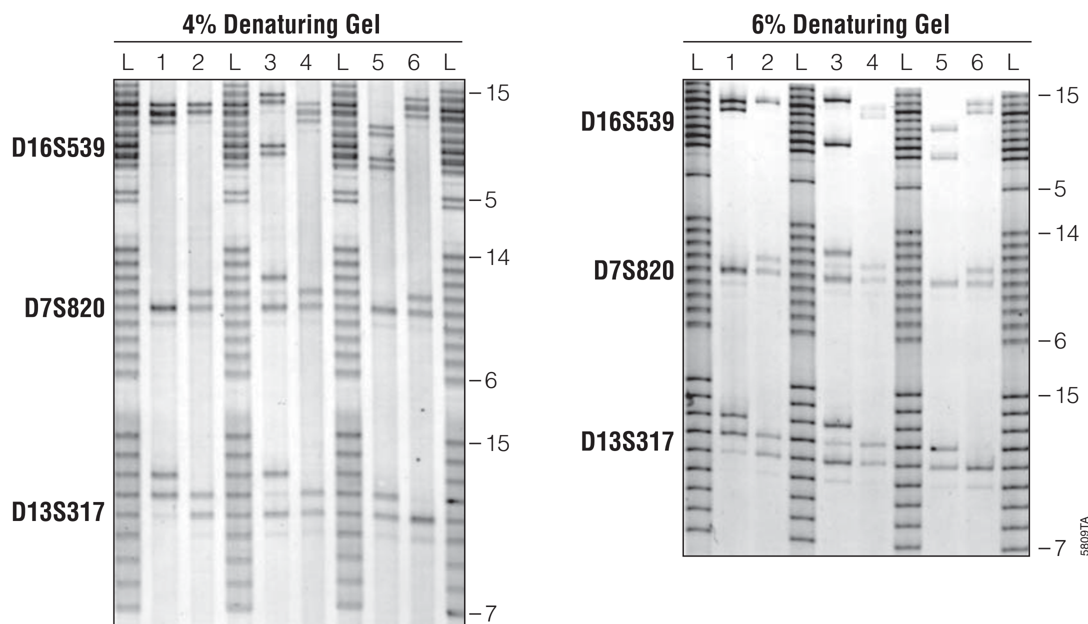

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Figure 2. ***GenePrint*® SilverSTR® III System.** Lanes 1–6 show amplification of 1ng of human DNA using the *GenePrint*® SilverSTR® III System. Amplification products were separated using a 4% denaturing polyacrylamide gel and a 6% denaturing polyacrylamide gel and were detected by silver stain analysis. Lanes labeled L contain the SilverSTR® Allelic Ladder Mix. Numbers to the right of each image indicate the smallest and largest number of tandem repeat units present in corresponding fragments of each allelic ladder. Note that for the locus D16S539 the strands of the individual alleles separate forming doublets. This results from sequence differences between the two complementary strands, which affect their relative migration. In a 6% gel, the doublets are closely spaced and do not interfere with interpretation. In a 4% gel, however, these doublets separate such that the top strand from one allele overlaps with the bottom strand of the next larger allele, requiring greater care during interpretation.

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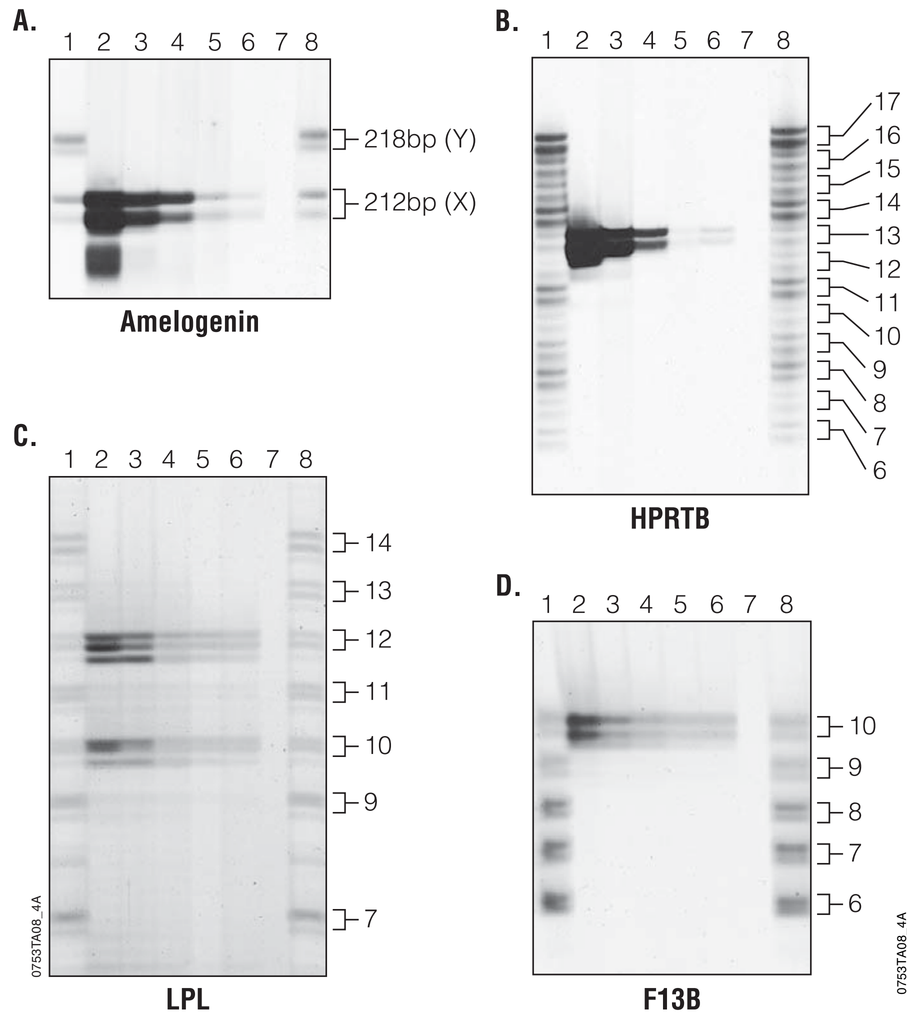

Figure 3. Amplification of varying concentrations of K562 template DNA at different STR loci and the Amelogenin locus. K562 DNA was amplified at the Amelogenin locus and various STR loci. The Perkin-Elmer model 480 thermal cycler was used with protocols 1 or 2 (Table 4). The use of protocol 7 does not produce the lowest fragment of each trio seen in the F13B and LPL products (data not shown). For each panel, lanes 1 and 8 contain the locus-specific allelic ladder; lanes 2–6 contain amplified K562 DNA using 250, 25, 5, 1 and 0.5ng of starting template, respectively; lane 7 contains a negative control amplification reaction (i.e., no template DNA). **Note:** The F13B Allelic Ladder has been updated to include alleles 5 through 11.

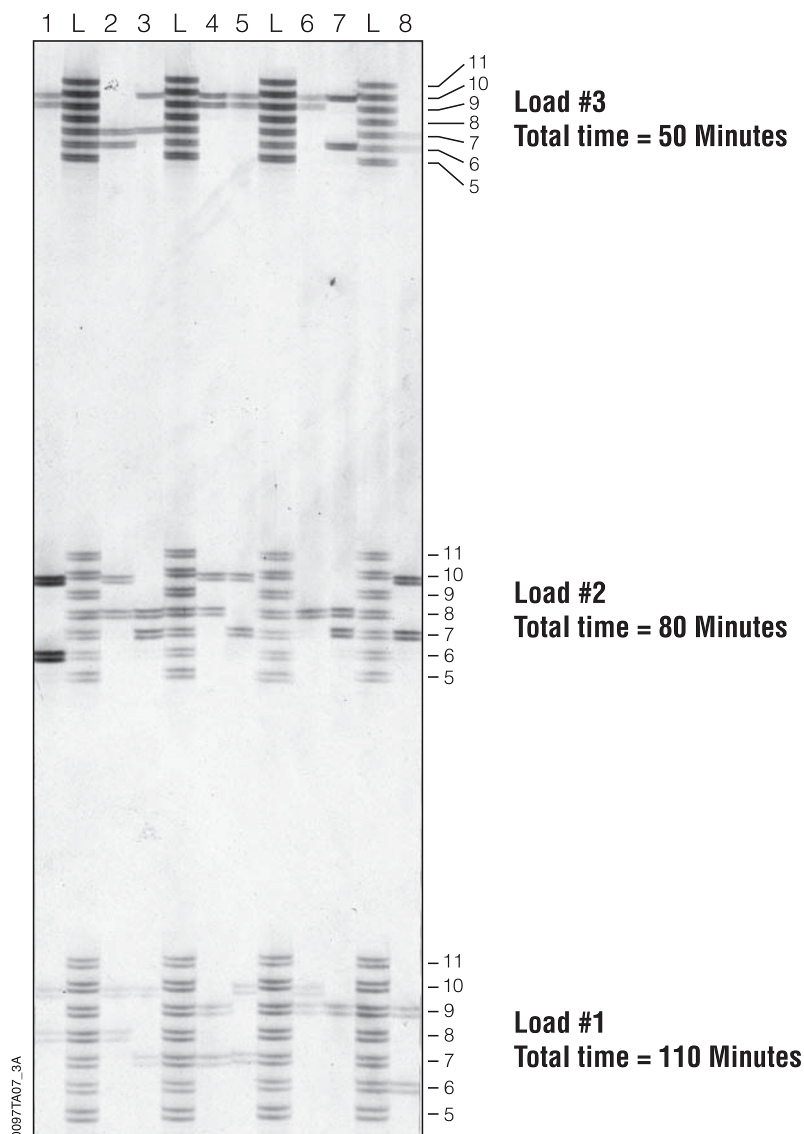

Figure 4. Sequential loading of STR locus TH01. For more efficient use of one 4% denaturing polyacrylamide gel, samples may be loaded at 30-minute intervals to obtain three times the amount of information when analyzing an individual STR system. Randomly selected DNA samples were amplified using the *GenePrint®* STR System—TH01 as described in this manual. Samples and ladders were loaded at three different times: load #1 (time 0), load #2 (30 minutes after load #1) and load #3 (60 minutes after load #1). Following load #3, samples were run for an additional 50 minutes. Separated products were detected by silver stain analysis. Lanes L, TH01 Allelic Ladder; lanes 1–8, amplified DNA from several individuals.

11. 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 no 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 exist in the DNA sample.
	Insufficient template DNA. Use the recommended amount of template DNA.
	Insufficient enzyme activity. Use the recommended amount of *Taq* DNA polymerase. Check the expiration date on the tube label.
	Wrong amplification program. Choose the correct amplification program for each locus.
	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 or tube problems. Review the thermal cycling protocols in Section 4. We have not tested other reaction tubes or thermal cyclers. Calibration of the thermal cycler heating block may be required.
	Primer concentration was too low. Use the recommended primer concentration. Mix well before use.
	Ice was not used to set up reactions. Set up the reactions on crushed ice. Very light allele intensity is obtained with some loci if ice is not used when setting up the reactions. The use of AmpliTaq Gold® DNA polymerase will also remedy this problem.
	Samples were not denatured before loading onto the gel. Be sure the samples are heated at 95°C for 2 minutes immediately prior to loading.
	Improper rinsing following staining. The rinse step was performed for more than 20 seconds. Longer rinses remove the silver deposited on the DNA. Rinse for a shorter time.
	Poor-quality water was used. Use ultrapure water (e.g., NANOpure®- or Milli-Q®-purified water) or double-distilled water.
Bands are fuzzy throughout the lanes	Poor-quality polyacrylamide gel. Prepare acrylamide and buffer solutions using high-quality reagents. Store acrylamide solutions in the dark.
	Electrophoresis temperature was too high. Run gel at lower temperature (40–60°C).

Symptoms	Causes and Comments
Background smearing within lanes	Poor-quality polyacrylamide gel. The amount of smearing may be reduced with high-quality polyacrylamide. **Note:** The allelic ladder for the D16S539 locus in the SilverSTR® III System shows some smearing.
	Sample overloading. Reduce the amount of template DNA used, or dilute the amplification reaction before mixing with loading buffer.
	BSA was used in amplification. The use of BSA is optional in the amplifications. BSA will stain with silver, resulting in a high-molecular-weight smear within the sample lane.
Extra bands visible in one or all of the lanes	Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipet tips, and change gloves regularly.
	Artifacts of STR amplification. PCR amplification sometimes generates artifacts that appear as faint bands one or four bases below an allele. Refer to Section 13.B for locus-specific information regarding this event.
	Artifacts of amplification with the *GenePrint*® SilverSTR® III System. Performance variation of thermal cyclers may cause extraneous bands to be generated outside of the allele range. The use of AmpliTaq Gold® DNA polymerase with this system may minimize or eliminate these extra bands. Alternatively, raising the annealing temperature to 62°C can also minimize or eliminate these bands. At annealing temperatures higher than 62°C, the amplification of D7S820 alleles may be compromised.
	Samples were not completely denatured. Heat denature the samples at 95°C for 2 minutes immediately prior to loading the gel.
Gel looks yellow after silver staining	Gel was left in the developer solution too long. Do not leave gel in developer solution for more than 5 minutes. Often, 2 minutes is sufficient.
High background on gel	Excess silver nitrate was present. Rinse the gel for 10 seconds in deionized water before adding developer solution.
	Development was too long. Stop the development reaction after the appearance of the alleles and ladders.
Gel adheres to both plates	Longer glass plate was contaminated with binding solution, or treatment of the longer glass plate with Gel Slick® solution was inadequate. Wipe excessive binding solution from the short glass plate. Exercise care to avoid contaminating the longer glass plate with binding solution. Ensure uniform coverage of the longer plate with Gel Slick® solution.

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12. References

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13. Appendix

13.A. Advantages of STR Typing

The *GenePrint*® STR Systems provide a rapid, non-radioactive method, which can be used to evaluate very small amounts (e.g., 1ng) of human DNA. The protocols detailed in this manual describe the use of silver staining (21) to detect the presence of amplified STR products following their separation by denaturing polyacrylamide gel electrophoresis. Information on detecting STR products by fluorescence methods is available at: **www.promega.com**

STR typing is more tolerant of the use of degraded DNA templates than other methods of individual identification because the amplification products are less than 400bp long, much smaller than the material detected with AMP-FLP (22) or VNTR (23) analysis. This format is also amenable to a variety of rapid DNA purification techniques.

In addition to these advantages, the STR loci chosen for inclusion in the *GenePrint*® Systems contain alleles of discrete and separable lengths. This allows the construction of allelic ladders, which contain fragments of the same lengths as several or all known alleles for the locus. Visual comparison between the allelic ladder and amplified samples of the same locus allows rapid and precise assignment of alleles. Results obtained using the *GenePrint*® STR Systems 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 (24).

13.B. Advantages of Using the Loci in the *GenePrint*® STR Systems

The STR loci and primers contained in the *GenePrint*® STR Systems (Tables 6 and 7) have been carefully selected to minimize artifacts, including those associated with *Taq* DNA polymerase, such as repeat slippage and terminal nucleotide addition as well as genetic artifacts called microvariant alleles. Repeat slippage (16–18), sometimes called “n–4 bands”, “stutter” or “shadow bands”, is due to the loss of a repeat unit during DNA amplification. The amount of this artifact observed depends primarily on the locus and the DNA sequence being replicated. We have chosen loci that exhibit little or no repeat slippage. The vWA locus is an exception, revealing as much as 10% stutter. This locus has been included primarily for its popularity in the forensic DNA-testing community.

Terminal nucleotide addition occurs when *Taq* DNA polymerase adds a nucleotide, generally adenine, to the ends of amplified DNA fragments in a template-independent manner (19,20). 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. Redefinition of the primer sequences and/or the addition of a final extension step of 60°C for 30 minutes to the amplification protocol can lead to essentially full terminal nucleotide addition (18).

Table 6. Locus-Specific Information.

STR Locus	Chromosomal Location	GenBank® Locus and Locus Definition	Repeat Sequence 5´→ 3´
Amelogenin[1]	Xp22.1–22.3 and Y	HUMAMEL, Human Y chromosomal gene for amelogenin-like protein	NA
CSF1PO	5q33.3–34	HUMCSF1PO, Human c-fms proto-oncogene for CSF-1 receptor gene	AGAT[2]
D16S539	16q24–qter	NA	AGAT[2]
D7S820	7q11.21–22	NA	AGAT[2]
D13S317	13q22–q31	NA	AGAT[2]
F13A01	6p24.3–p25.1	HUMF13A01, Human coagulation factor XIII a subunit gene	AAAG[2]
F13B	1q31–q32.1	HUMBFXIII, Human factor XIII b subunit gene	AAAT[2]
FESFPS	15q25–qter	HUMFESFPS, Human c-fes/fps proto-oncogene	AAAT[2]
HPRTB	Xq26	HUMHPRTB, Human hypoxanthine phosphoribosyl-transferase gene	AGAT[2]
LPL	8p22	HUMLIPOL, Human lipoprotein lipase gene	AAAT[2]
TH01	11p15.5	HUMTH01, Human tyrosine hydroxylase gene	AATG[2]
TPOX	2p25.1–pter	HUMTPOX, Human thyroid peroxidase gene	AATG[2]
vWA (formerly vWF)	12p12–pter	HUMVWFA31, Human von Willebrand factor gene	AGAT[2]

NA = not applicable.

[1]Amelogenin 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.

[2]Repeat sequences represent all four possible permutations (e.g., AGAT is used for AGAT, GATA, ATAG or TAGA). The first alphabetic representation of the repeat (e.g., AGAT) is used according to the precedent of Edwards *et al*. (2). The published article, "DNA Guidelines: Further Report of the DNA Commission of the ISFH Regarding the use of Short Tandem Repeat Systems" (25) describes different rules for STR allele nomenclature. Allele designations for all listed loci are identical using both methods except for the locus F13B. In this case, alleles are one repeat unit larger when using the method described by the ISFH. For this locus, the community will have to decide whether to follow the new nomenclature or maintain the Edwards nomenclature to avoid confusion. The DNA Commission of the ISFH states "If a repeat designation of a commonly used STR system does not follow these guidelines, the established nomenclature for the sequence can continue to be used to avoid new confusion".

Table 7. Additional Locus-Specific Information.

STR Locus	Allelic Ladder Size Range[1] (bases)	STR Ladder Alleles (# of repeats)[2]	Other Known Alleles[3] (# of repeats)	K562 DNA Allele Sizes (# of repeats)	Comments
Amelogenin[4]	212–218	NA	None	212,212	1,2
CSF1PO	295–327	7,8,9,10,11, 12,13,14,15	6	10,9	1
D16S539	264–304	5,8,**9**,10,11, 12,**13**,14,15	None	12,11	1
D7S820	215–247	6,7,8,9,10, 11,12,13,14	None	11,9	1
D13S317	165–197	7,8,9,10,11, 12,13,14,15	None	8,8	1
F13A01	283–331	4,5,6,7,8,9,11, 12,13,14,15,16	3.2,10[5]	5,4[6]	1,3
F13B	169–189	6,7,8,9,10,11	12	10,10	1
FESFPS	222–250	7,8,9,10,11,12,13,14	None	12,10	1
HPRTB	259–303	6,7,**8**,9,10,**11**, 12,13,**14**,15,16,**17**	None	13,13	1
LPL	105–133	7,9,10,11,12,13,14	8	12,10	1
TH01	179–203	5,6,7,8,9,10,11	9.3	9.3,9.3	1,4
TPOX	224–252	6,7,8,9,10,11,12,13	None	9,8	1
vWA (formerly vWF)	139–167	13,14,15,16, 17,18,19,20	11,21	16,16	1

NA = not applicable.

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

[2]Alleles in bold are present in greater amounts than other alleles. This simplifies interpretation.

[3]Alleles that represent <0.2% of the population may not be listed in this table.

[4]Amelogenin 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.

[5]Allele 10 (307 bases) is not included because it is rare and its exclusion creates a gap that simplifies interpretation of the allelic ladder (26,27).

[6]F13A01 allele 5 appears more intense than allele 4 in the K562 control sample. The K562 strain contains an unusual number of chromosomes, and some are represented more than twice per cell. It is hypothesized that in this strain the allele 5 version of chromosome 6 is present twice, while the allele 4 version of chromosome 6 is present only once.

Table 8. Multiplex System Information.

GenePrint® STR Multiplex Cat.#	Component Loci	Allelic Ladder Size Range (bases)	STR Ladder Alleles (number of repeats)	Other Known Alleles[1]	K562 DNA Allele Sizes	Comments
"CTT triplex" [2] (DC6000 and DC6001)	CSF1PO	295–327	7,8,9,10,11, 12,13,14,15	6	10,9	1,5
	TPOX	224–252	6,7,8,9,10,11,12,13	None	9,8	1,5
	TH01	179–203	5,6,7,8,9,10,11	9.3	9.3,9.3	1,4,5
"FFv triplex" (DC6030 and DC6031)	F13A01	283–331	4,5,6,7,8,9,10, 11,12,13,14,15,16	3.2,10	5[3],4	1,3,5
	FESFPS	222–250	7,8,9,10,11,12,13,14	None	12,10	1,5
	vWA	139–167	13,14,15,16, 17,18,19,20	11,21	16,16	1,5
"Silver-STR® III triplex" (DC6450 and DC6451)	D16S539	264–304	5,8,9,10,11, 12,13,14,15	None	12,11	1,5
	D7S820	215–247	6,7,8,9,10, 11,12,13,14,	None	11,9	1,5
	D13S317	165–197	7,8,9,10,11, 12,13,14,15	None	8,8	1,5

[1]Alleles that represent <0.2% of the population may not be listed in this table.

[2]The *GenePrint*® Sex Identification System—Amelogenin primers may be combined with the CTT primers to allow simultaneous amplification of all four loci as described in Section 4.B. The results provide information regarding the gender of the individual who contributed the DNA sample, as well as the STR information. Ordering information for the Amelogenin system may be found in Section 13.I.

[3]F13A01 allele 5 appears more intense than allele 4 in the K562 control sample. The K562 strain is known to contain an unusual number of chromosomes, and some are represented more than twice per cell. It is hypothesized that in this strain the allele 5 version of chromosome 6 is present twice while the allele 4 version of chromosome 6 is present only once.

Comments on Tables 7 and 8

1. PCR amplification sometimes generates artifacts that appear as faint bands below the alleles. These products probably result from a process known as slippage, commonly observed in PCR amplification of regions that contain tandem repeats of short sequences (16–18). This characteristic is most pronounced with the vWA locus.

2. A strong extra band may be observed below the 212bp Amelogenin allele when more than 25ng of template DNA is amplified.

3. Locus F13A01 has a common allele 3.2. It contains 4 copies of the repeat but has a 2-base deletion in the region flanking the repeat (26,27).

4. Locus TH01 has a common 9.3 allele (9). A one-base deletion is present in the allele that contains 10 repeats. Note that reference 9 refers to this allele as 10–1 rather than 9.3. This allele was renamed 9.3 at the ISFH Conference in Venice, Italy, in October of 1993.

5. A background haze of silver stain is sometimes seen in the region in and above the allelic ladder.

13.C. Power of Discrimination

Table 9 shows the matching probability (28) for the multiplex *GenePrint*® STR Systems in various populations. When taken together, the triplexes described in this manual produce matching probabilities ranging from 1 in 1,030,000,000 in Caucasian-Americans to 1 in 5,180,000,000 in 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 a tested man (29). The typical PIs for the multiplex *GenePrint*® STR Systems are shown in Table 10. The three triplexes together give typical paternity indices exceeding 500 in each group, enough to satisfy routine requirements for paternity determination.

An alternative calculation used in paternity analyses is the power of exclusion (29). This value, calculated for the combined triplexes, exceeds 0.9985 in all populations tested (Table 11).

Table 9. Matching Probability of Various Populations.

	Matching Probability		
STR System	**African-American**	**Caucasian-American**	**Hispanic-American**
CTT triplex (CSF1PO, TPOX, TH01)	1 in 1,590	1 in 435	1 in 549
FFv triplex (F13A01, FESFPS, vWA)	1 in 2,828	1 in 927	1 in 1,343
SilverSTR® III triplex (D16S539, D7S820, D13S317)	1 in 1,152	1 in 2,552	1 in 2,493
All 3 triplexes (9 loci)	1 in 5.18×10^9	1 in 1.03×10^9	1 in 1.84×10^9

Table 10. Typical Paternity Indices of the Multiplex *GenePrint*® STR Systems in Various Populations.

	Typical Paternity Index		
STR System	**African-American**	**Caucasian-American**	**Hispanic-American**
CTT triplex (CSF1PO, TPOX, TH01)	10.2	6.8	5.2
FFv triplex (F13A01, FESFPS, vWA)	16.0	9.8	7.8
SilverSTR® III triplex (D16S539, D7S820, D13S317)	7.6	7.7	14.1
All 3 triplexes (9 loci)	1233	521	563

Table 11. Power of Exclusion of the *GenePrint*® STR Systems in Various Populations.

STR System	Power of Exclusion		
	African-American	Caucasian-American	Hispanic-American
CTT triplex (CSF1PO, TPOX, TH01)	0.906	0.869	0.830
FFv triplex (F13A01, FESFPS, vWA)	0.938	0.904	0.881
SilverSTR® III triplex (D16S539, D7S820, D13S317)	0.877	0.880	0.929
All 3 triplexes (9 loci)	0.9993	0.9985	0.9986

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13.D. 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 (30). 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.

For applications requiring human-specific DNA quantification, the AluQuant® Human DNA Quantitation System (Cat.# DC1010) has been developed (31).

The DNA IQ™ System and AluQuant® Human DNA Quantitation System have been fully automated on the Beckman Coulter Biomek® 2000 Laboratory Automation Workstation (32). For information about automation of laboratory processes on Beckman Coulter or other workstations, contact your local Promega Branch Office or Distributor (contact information available at: **www.promega.com/worldwide/**) or e-mail: **genetic@promega.com**

Note: For stains from blood and saliva, scientists at the FBI Academy have suggested an alternative method for DNA extraction (see reference 33).

13.E. Agarose Gel Electrophoresis of Amplification Products (Optional)

This procedure is optional if PCR is routinely performed in your laboratory. Agarose gel electrophoresis can be 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 13.F.)

- TAE 1X buffer
- agarose
- 5X loading solution
- ethidium bromide solution, 0.5µg/ml

Ethidium bromide is a powerful mutagen. Wear gloves at all times, and use a mask when weighing out ethidium bromide powder.

1. Prepare a 2% agarose gel (approximately 150cm^2) 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 10µl of each amplified sample with 2.5µl 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 5X loading solution (prepared in Step 3).
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 0.5µg/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. Using a UV transilluminator (302nm), photograph the gel (e.g., with Polaroid® 667 film).

   Note: When analyzing the data, do not be alarmed by 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 reaction.

13.F. Composition of Buffers and Solutions

0.5% acetic acid in 95% ethanol

Add 1ml of glacial acetic acid to 199ml of 95% ethanol.

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 500µl for one acrylamide gel solution (75ml). Store the remaining volume in 500µl aliquots at –20°C.

developer solution

3ml	37% formaldehyde (H_2CO)
400µl	10mg/ml sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)
2L	deionized water
60g	sodium carbonate (Na_2CO_3)

Sodium carbonate must be ACS grade. We have confirmed quality with material from Fisher Scientific (Fisher Scientific Cat.# S263-500). Results may vary depending on source.

Prepare the solution, and chill to 10°C before use. Use only high-quality deionized water and sodium carbonate. Prepare fresh before each use.

0.5M EDTA (pH 8.0) stock

186.1g	$Na_2EDTA \cdot 2H_2O$

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

ethidium bromide stock solution

Add 1g of ethidium bromide to 100ml of deionized water. Stir on a magnetic stirrer until dye is dissolved. Wrap the container in aluminum foil, or transfer to a dark bottle. Store at room temperature.

GoldST*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

5X loading solution

5%	Ficoll® 400
0.1%	bromophenol blue
0.1%	xylene cyanol
100mM	EDTA ($Na_2EDTA \cdot 2H_2O$)
10mM	Tris-HCl (pH 7.5)

fix/stop solution (10% acetic acid)

200ml	glacial acetic acid
1,800ml	deionized water

sodium thiosulfate solution (10mg/ml)

Add 5g of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) to 500ml of deionized water.

staining solution

2g	silver nitrate ($AgNO_3$)
3ml	37% formaldehyde (H_2CO)
2,000ml	deionized water

STR 2X Loading Solution

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

13.F. Composition of Buffers and Solutions (continued)

STR 10X Buffer

500mM	KCl
100mM	Tris-HCl (pH 9.0) at 25°C
15mM	$MgCl_2$
1%	Triton® X-100
2mM	each dNTP

50X TAE 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.

0.5X TBE buffer

Add 50ml of 10X TBE to 950ml of deionized water.

10X TBE buffer

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

Dissolve the Tris base and EDTA in 800ml of deionized water. Add slightly less than the total amount of boric acid. Mix until completely dissolved, check the pH, and adjust to 8.3 with boric acid. 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 ($Na_2EDTA \bullet 2H_2O$)

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

13.G. Population Data

Allele frequencies for African-Americans, Caucasian-Americans and Hispanic-Americans were generated as part of a collaborative effort between Genetic Design, Inc. (Greensboro, NC), and Promega Corporation (34). This population data can be found at: **www.promega.com/techserv/apps/hmnid/referenceinformation/popstat/custstat_Allelefreq.htm**

13.H. Organizational Sheets

Sample Preparation

Tube Number							
Sample ID	negative control						
Sample Conc. (ng/µl)	–						
Sample (µl)/reaction	0						
Sterile Water (µl)	2.5						

Tube Number							
Sample ID							
Sample Conc. (ng/µl)							
Sample (µl)/reaction							
Sterile Water (µl)							

Tube Number							
Sample ID							
Sample Conc. (ng/µl)							
Sample (µl)/reaction							
Sterile Water (µl)							

Tube Number							
Sample ID							
Sample Conc. (ng/µl)							
Sample (µl)/reaction							
Sterile Water (µl)							

Tube Number							
Sample ID							
Sample Conc. (ng/µl)							
Sample (µl)/reaction							
Sterile Water (µl)							

Tube Number							
Sample ID							
Sample Conc. (ng/µl)							
Sample (µl)/reaction							
Sterile Water (µl)							

Master Mix Preparation

Date:

Name:

GenePrint® STR Systems Locus =

Reaction volume (sample + master mix) = 25µl

Number of reactions = ________

Master Mix Component	Lot Number	Volume Per Sample (µl)	×	Number of Reactions	=	Final Volume (µl)
sterile water		17.45 for monoplex 17.35 for quadriplex	×		=	
STR 10X Buffer[1]		2.50	×		=	
_____ 10X Primer Pair		2.50	×		=	
Taq DNA polymerase (5u/µl)[2]		0.05 for monoplex 0.15 for quadriplex	×		=	
				total volume	=	

[1]If using AmpliTaq Gold® DNA polymerase, use the GoldST*R 10X Buffer (Cat.# DM2411, available separately) instead of the STR 10X Buffer.

[2]The volume given assumes a *Taq* DNA polymerase concentration of 5u/µl. For different enzyme concentrations, the volume of enzyme added must be adjusted accordingly.

To assemble reactions, add 2.5µl DNA to each tube containing 22.5µl of master mix.

Thermal Cycling Profile

Perkin-Elmer Thermal Cycler Model Number:

Annealing Temperature:

File Number:

Full Program Description:

______cycles:

_______°C _______ minutes
_______°C _______ minutes
_______°C _______ minutes

______cycles:

_______°C _______ minutes
_______°C _______ minutes
_______°C _______ minutes

Hold: 4°C indefinitely

Experiment

Date:
Name:

Electrophoresis

Pre-run:	____________ minutes		
Starting time:	____________	Stopping time:	____________
Watts:	____________	Watts:	____________
Milliamps:	____________	Milliamps:	____________
Voltage:	____________	Voltage:	____________

Notes

Gel Number:

Lane	Sample #	Description
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		

Lane	Sample #	Description
1		
2		
3		
4		
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6		
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25		

13.I. Related Products

Fluorescent STR Multiplex Systems

Product	Size	Cat.#
PowerPlex® 1.1 System	100 reactions	DC6091
	400 reactions	DC6090
PowerPlex® 2.1 System	100 reactions	DC6471
	400 reactions	DC6470
PowerPlex® 1.2 System	100 reactions	DC6101
PowerPlex® 16 System	100 reactions	DC6531
	400 reactions	DC6530
GenePrint® GammaSTR® Multiplex (Fluorescein) D16S539, D7S820, D13S317, D5S818	100 reactions	DC6071
	400 reactions	DC6070
GenePrint® Fluorescent STR CSF1PO, TPOX, TH01, vWA Multiplex (Fluorescein)	100 reactions	DC6301
	400 reactions	DC6300
GenePrint® Fluorescent STR F13A01, FESFPS, F13B, LPL Multiplex (Fluorescein)	100 reactions	DC6311
	400 reactions	DC6310

Not for Medical Diagnostic Use.

Accessory Components

Product	Size	Cat.#
GoldST*R 10X Buffer	1.2ml	DM2411
Mineral Oil	12ml	DY1151
Nuclease-Free Water	50ml (2 × 25ml)	P1193

Sample Preparation Systems

Product	Size	Cat.#
DNA IQ™ System	100 reactions	DC6701
	400 reactions	DC6700
Differex™ System*	50 samples	DC6801
	200 samples	DC6800
Slicprep™ 96 Device	10 pack	V1391

*Not for Medical Diagnostic Use.

Polyacrylamide Gel Electrophoresis Reagents

Product	Size	Cat.#
Ammonium Persulfate	25g	V3131
TBE Buffer, 10X	1L	V4251

Printed in USA.
Revised 12/10

Urea	1kg	V3171
Blue Dextran Loading Solution	3ml	DV4351

[a]STR loci are the subject of U.S. Pat. No. RE 37,984, German Pat. No. DE 38 34 636 C2 and other patents issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, e.V., Germany. The development and use of STR loci are covered by U.S. Pat. No. 5,364,759, Australian Pat. No. 670231 and other pending patents assigned to Baylor College of Medicine, Houston, Texas.

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.

[b]U.S. Pat. No. 5,843,660, Australian Pat. No. 724531, Korean Pat. No. 290332, Singapore Pat. No. 57050 and Japanese Pat. No. 3602142 have been issued to Promega Corporation for multiplex amplification of STR loci. Other patents are pending.

[c]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 and Japanese Pat. No. 3602142 have been issued to Promega Corporation for multiplex amplification of STR loci. Other patents are pending.

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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.