

Current Protocols in Human Genetics
Unit 14.8

Short Tandem Repeat Analysis for Human Identity Testing

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Abstract:

Short tandem repeat (STR) typing has become the workhorse of modern forensic DNA analysis. The most common form of STR typing uses laser-induced fluorescence detection of dye-labeled polymerase chain reaction (PCR) products following capillary electrophoresis (CE) size-based separation. This unit describes the techniques and marker systems most widely used around the world in constructing criminal DNA databases and conducting forensic casework. In addition, these same STR loci and multiplex PCR assays are commonly used for paternity testing, missing persons and mass disaster investigations. Both autosomal and Y-chromosome STRs are discussed in the context of their various applications. Interpretation issues surrounding degraded or mixed DNA specimens as well as tri-allelic patterns and variant alleles are also illustrated. Finally future technologies and DNA marker systems are briefly explored relative to the state-of-the-science today.

Information about accompanying diskette:

Short Tandem Repeat Analysis for Human Identity Testing

The exquisite sensitivity of the polymerase chain reaction (PCR) now permits DNA analysis from samples containing as little as a single cell (Findlay et al., 1997). This capability has extended the application of DNA profiling methods to new areas in forensic science including the ability to obtain accurate results from fingerprints left on touched objects (van Oorschot et al., 1997) or badly damaged samples obtained from a mass disaster (Whitaker et al., 1995).

The most commonly used PCR-based typing method today involves short tandem repeats, or STRs (Butler 2001). STRs are regions of DNA where short sequences, usually between 2-6 basepairs (bp), are repeated side-by-side. Since the early 1990s, there are tens of thousands of these STRs that have been discovered to occur within and between genes along human chromosomes. Due to the fact that they do not appear to disrupt normal cellular functions, STRs can easily mutate giving rise to a high degree of variability between individuals. A dozen or more alleles are possible for many STR loci.

A small subset of available STRs have been selected and standardized for use in human identity testing and criminal DNA databasing. In late 1997 the Federal Bureau of Investigation (FBI) chose 13 STR loci for inclusion in the U.S. Combined DNA Index System (CODIS). These loci CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11 form the basis of most criminal DNA databases around the world (Budowle et al., 2000). Commercially available kits enable accurate and relatively rapid characterization of STR genotypes.

The goal of this unit is to describe methods involving commercial kits for analyzing STR loci commonly used in human identity testing. An overview of the process for STR analysis is shown

in **Figure 14.8.1**. Information on various extraction and quantification methods that provide DNA samples for PCR amplification can be found in *Unit 14.3*. Basic Protocol 1 focuses on PCR amplification of STR markers, Basic Protocol 2 provides descriptions of methods for separation and detection of STR amplicons, and Basic Protocol 3 discusses software used for genotyping STR amplicons. Finally in the commentary section critical parameters are considered along with some data interpretation issues, time considerations, and possible future trends.

<Insert Figure 14.8.1 *overall process to obtain STR typing results* near here>

Basic Protocol 1

AMPLIFICATION OF STR MARKERS: Identifiler kit

The commercial kit Identifiler™ from Applied Biosystems enables co-amplification of 15 STR loci and the sex-typing marker amelogenin with four different dye labels (Applied Biosystems, 2001a). The protocol for use of Identifiler is described here as it represents other STR kits that amplify fewer loci with only 1-3 different dye colors. Several additional protocols are included for amplification of PowerPlex® 16 (**Alternative Protocol 1**), and Y-PLEX™ 12 (**Alternative Protocol 2**).

Materials

Thermal cycler (e.g., GeneAmp 9600 or 9700 in 9600-emulation mode)
Identifiler STR kit (Applied Biosystems, P/N 4322288): includes AmpliTaq Gold, primer mix, and PCR components such as dNTPs, MgCl₂, and buffer
0.20-mL thin-walled PCR tubes
1.5-mL sterile tube
DNA extract of appropriate biological specimens that have been previously quantified, and accompanying positive and negative controls (Unit 14.3)
AmpFlSTR Identifiler PCR Amplification Kit User's Manual

PCR Setup

1. Label PCR tubes with sample names.
2. For each 25- μ L reaction, add 10.5 μ L PCR reaction mix, 0.5 μ L AmpliTaq Gold DNA polymerase, and 5.5 μ L Identifiler primer set.

NOTE: If more than one sample is being prepared in parallel, it is helpful to make a PCR master mix in a 1.5-mL sterile tube that can then be equally dispensed to all tubes in order to maintain consistency between samples.

3. Vortex this Identifiler master mix to insure that it is homogeneous and dispense 15 μL to each PCR tube.
4. Add 10 μL of extracted DNA to each PCR tube containing the Identifiler master mix so that a total amount of 0.5-1.25 ng DNA is present (0.05-0.125 ng/ μL). Alternatively, deionized water or TE buffer can be added to fill the remaining volume if a more concentrated sample is used.
5. Setup positive and negative control samples in addition to the DNA samples under testing. The positive control can be a fixed amount, such as 1.0 ng, of the 9947A kit control sample and is used to verify that PCR components are working properly. The negative control can be 10 μL of deionized water or TE buffer in place of DNA and is used to verify that PCR components are not contaminated with extraneous human DNA.
6. Cap PCR tubes.
7. Place PCR tubes into the thermal cycler.
8. Initiate a thermal cycling program that performs the temperature steps shown in Table 14.8.1. If a GeneAmp 9700 thermal cycler is used, make sure that the temperature ramp speeds are set for 1 $^{\circ}\text{C}/\text{second}$ (9600-emulation) rather than 4 $^{\circ}\text{C}/\text{second}$ (9700-default).

<Insert Table 14.8.1 *thermal cycling for Identifiler kit* near here >

Basic Protocol 2

SEPARATION AND DETECTION OF STR AMPLICONS: Use of ABI 310

The ABI 310 Genetic Analyzer is a capillary electrophoresis instrument that permits separation and detection of fluorescently labeled DNA products in a single capillary (Applied Biosystems 2001b). PCR products from STR amplifications generated in Basic Protocol 1 are diluted into deionized formamide and mixed with an internal size standard. These samples tubes are then covered with a rubber septum and loaded in an autosampler tray that can hold either 48 or 96 samples. Before each run, the capillary is filled with a polymer solution that enables size-based

separations during electrophoresis. The dye labeled-PCR products are injected into the polymer-filled capillary when the autosampler raises an individual sample tube onto the capillary and the cathode and an electric voltage is applied for a few seconds. The PCR products are then separated as they pass through the entangled polymer network inside the capillary under an electric field generated with typically 15,000 volts. As the dye-labeled amplicons pass an Argon ion laser beam fixed approximately 36 cm from the injection end of the capillary, a charged-coupled device (CCD) camera records the fluorescence wavelength and scan number (data point from start of run). This information is recorded in a collection file that becomes the electropherogram, which is then processed to determine the STR genotypes present in the sample (see **Basic Protocol 3**). Scan numbers for STR alleles are converted into DNA size through comparison to peak positions of the internal size standard. Dye colors are resolved through the use of a mathematical matrix, which is usually generated prior to analyzing the STR samples. GeneScan software is used to classify peaks and to separate the peaks by color through applying the matrix. Genotyper software is then used to compare peak bp sizes from a sequenced allelic ladder (provided with the STR kit) to the sizes of each STR allele to generate an allele call. The allelic ladder is run under the same electrophoretic conditions usually within a short time period from the other sequentially processed samples. An allelic ladder may be run as part of the same batch as the samples being typed or just before or after the sample set.

Materials (with part numbers from Applied Biosystems, Foster City, CA)

- 48-tube sample tray, P/N 402867
- Genetic Analyzer sample tubes (0.5mL), P/N 401957
- Genetic Analyzer septa for 0.5mL sample tubes, P/N 401956
- 310 capillaries, 47cm x 50 μ m i.d., P/N 402839
- 1.0mL glass syringe, P/N 4304471
- Genetic Analyzer buffer vials (4.0mL), P/N 401955
- POP-4 Performance Optimized Polymer, P/N 402838
- 10X Genetic Analyzer Buffer, P/N 401884
- 15-mL falcon tube

1.5 mL sterile tube
Methanol and Kimwipes or isopropanol wipes
GeneScan-500 LIZ size standard, P/N 4322682
Hi-Di formamide, P/N 4311320
Matrix Standard Set DS-33 (6-FAM, VIC, NED, PET, and LIZ), P/N 4318159
ABI Prism 310 Genetic Analyzer User's Manual

Instrument Setup and Matrix Definition

1. Turn on ABI Prism 310 Genetic Analyzer instrument.
2. Turn on computer.
3. Clean the pump block (ABI 310 User Manual, page 4-8).
4. Put capillary into ABI 310 instrument according to manufacturer's instructions (ABI 310 User Manual, pages 4-11 to 4-15). Clean the detection window with an isopropanol wipe or use methanol on a Kimwipe.

NOTE: A clean window is essential to eliminate light scatter and lower the fluorescent background of the electropherogram. It is also important to keep the ends of the capillary in liquid after the capillary has been filled with buffer and polymer solution to prevent crystal formation that will plug the inlet of the capillary.

5. Fill syringe with POP-4 polymer that has equilibrated to room temperature (see ABI 310 User Manual, page 4-22).
6. Dilute 10X buffer to 1X with deionized water by mixing 1.5-mL 10X buffer and 13.5-mL deionized water in a 15-mL falcon tube (see ABI 310 User Manual, page 4-23).
7. Fill the anode buffer reservoir to the red line with 1X Genetic Analyzer buffer.
8. Fill cathode buffer vial with 1X buffer and place in position 1 on the autosampler tray (see ABI 310 User Manual, page 4-23).
9. Fill a Genetic Analyzer buffer vial up to the black line with deionized water and place vial in position 2 of the autosampler tray.
10. Clip the lid off of a 1.5 mL tube, fill with deionized water, and place in position 3 of the autosampler tray. This tube will act as a waste vial. Since this tube is not covered, the water will evaporate over time and need to be replaced so that the inlet of the capillary always comes in contact with liquid when in the waste vial position.

11. Setup five different matrix samples in 0.5-mL tubes by mixing 19- μ L Hi-Di formamide with 1- μ L of each dye labeled sample in the DS-33 matrix standard set.
12. Cap 0.5-mL tubes with septa.
13. Load autosampler tray with the five matrix standard samples.
14. Follow instructions in ABI 310 manual to create a 5x5 matrix (ABI 310 User Manual, pages 3-42 to 3-45). This multicomponent matrix will be used to mathematically deconvolute overlapping dye emissions and produce clean monochromatic peaks to processed electropherograms.

Sample Preparation for Analysis

1. Setup an appropriate number of samples tubes with the PCR samples amplified in Basic Protocol 1. Add one extra tube for an allelic ladder sample.
2. Mix 0.3- μ L GS500 LIZ size standard with 19- μ L Hi Di formamide.

NOTE: If more than one sample is being prepared in parallel, it is helpful to make a master formamide/size standard mix in a 1.5-mL sterile tube that can then be equally dispensed to all tubes in order to maintain consistency between samples. Add one extra volume for every ten samples to correct for volume lost in pipetting.

3. Add 19- μ L Hi-Di formamide/size standard mix to each 0.5-mL tube.
4. Carefully pipet 1- μ L of each Identifiler sample amplified in Basic Protocol 1, or the allelic ladder from the Identifiler kit, into the 0.5-mL analysis tubes containing the Hi-Di formamide.
5. Cap tubes with septa. It is helpful to mark tubes or the tops of the septa with sample names or sequential numbers using an extra fine point Sharpie marker to avoid sample mix-ups in the tray.
6. Flick tubes with forefinger to mix contents. Gently tap the tubes on a lab bench to bring liquid droplets to bottom of the tube. Make sure that there are no bubbles after mixing and that the liquid extends to the bottom of the tube.

NOTE: Our laboratory has observed in running thousands of samples that there is no need to heat denature and snap cool samples as is typically done in many protocols. Simple addition of PCR product to deionized formamide is enough to enable effective denaturation of samples.

7. Place samples into 48-tube tray carefully noting the position of each sample. Samples should be added across the tray (A1, A3, A5, A7, A9, A11, B2, etc.).
8. Place tray onto the ABI 310 Autosampler and close instrument doors.

Setup Sample Data Collection

1. Launch the 310 Data Collection software (version 3.0.0). Alternatively, 310 Data Collection Software v2.1 or above can be used with a Mac to enable five-dye data collection. The steps listed below are virtually the same for PC or Mac versions.
2. Go to the *Window: Manual Control* section and select *Temperature Set* from the drop down menu. Set the temperature to 60 °C and click Execute. This will enable the instrument to heat up while the sample names and run conditions are being entered.
3. Click on *File: New: GeneScan Smpl Sheet 48 Tube* to open a new sample sheet.
4. Select 5-dye collection in upper right hand corner.

NOTE: The use of 5-dye data collection is specific to the Identifiler kit. Alternative Protocols 1 and 2 use 4-dye data collection.

5. Enter names of samples in the Sample Name column for positions corresponding to tray positions.
6. Copy and paste sample names to the Sample Info column so that each dye color has the sample name listed. Each sample needs to have a unique sample number or name in the Sample Info column for automated allele calling with Genotyper software (see below). The allelic ladder sample must have the word “LADDER” in the Sample Info column. Make sure that the Std column has a diamond in the orange dye lane for the GS500 LIZ size standard.
7. Save the Sample Sheet.
8. Click on *File: New: GeneScan Injection List* to setup the sample injection order and electrophoretic conditions for sample processing.
9. From the drop down menu, load the Sample Sheet previously saved. The Tube & Sample Name column should be populated with the information recorded from the Sample Sheet.
10. Select GS STR POP4 (1 mL) G5 for the Module column on the first sample. Use Ctrl+D to fill down the column with the same module information.

11. Check the Inj. Secs column to ensure that it is set at 5 and the Inj. KV column that it is set at 15.0. This will enable a 5 second electrokinetic injection of the DNA samples with 15,000 volts.

NOTE: If a sample is overloaded and the signal is off-scale, the time can be shortened to 2-3 seconds instead of 5. Likewise, if a greater signal is desired from a weakly amplifying sample, then the injection time can be increased to 7-10 seconds to load more DNA onto the capillary. A series of samples should be run with higher and lower injection times as part of validation to ensure that no other artifacts are seen.

12. The Run kV column should be 15.0, the Run °C column 60, and the Run Time 28 minutes.

NOTE: Run times may need to be lengthened by a few minutes to ensure that DNA fragments up to at least the 450 bp peak have passed the detector and been recorded in the electropherogram.

13. The Matrix File column does not have to be filled prior to data collection but can be if the matrix has already been created as described previously.

14. Enter the operator name or initials.

15. Click the Run button to initiate the run. It may take several minutes to begin execution of the run depending on the instrument temperature, which must be 60 °C before it can begin.

NOTE: Watch the first injection to make sure that a syringe leak does not occur. The ABI 310 will pause and require manual intervention to continue a run if a leak is detected while trying to fill the capillary with polymer. If this is a problem, tighten the capillary fitting that connects the capillary to the pump block. The run current should be approximately 8 μ A with 15 kV. If it is zero or is fluctuating by more than 1-2 μ A, then the capillary is likely plugged and may need to be replaced.

Basic Protocol 3

DATA ANALYSIS AND STR GENOTYPING

GeneScan® and Genotyper® are software programs available from Applied Biosystems to enable DNA fragment analysis including STR alleles. These programs were originally written for Macintosh computers but more recently have been adapted to run on Microsoft Windows NT and

Windows 2000 platforms. More recently, Applied Biosystems has developed another program called GeneMapper[®] that combines the functions of GeneScan and Genotyper.

Once data has been collected on the ABI 310 Genetic Analyzer (**Basic Protocol 2**) or the ABI 3100 multi-capillary Genetic Analyzer (**Alternative Protocol 3**), the raw data is converted into the appropriately colored peaks and STR genotype information using the GeneScan and Genotyper programs. GeneScan performs three primary functions: (1) it calls peaks based on threshold values specified by the user, (2) it separates the peaks into the appropriate dye color based on a matrix file, and (3) it sizes the STR allele peaks based on an internal size standard labeled with a different colored dye that is run in every sample. The Genotyper software program takes GeneScan data and converts the sized peaks into genotype calls. Genotyping is performed by comparison of allele sizes in an allelic ladder to the sample alleles. The manufacturer of a particular STR kit normally provides Genotyper macros in order to make the allele calls from the allelic ladders. These macros can be designed to filter out stutter peaks that can interfere with sample interpretation (see **Commentary**).

Materials

GeneScan software version 3.7, Applied Biosystems
Genotyper NT software version 3.7, Applied Biosystems
ABI Prism Genotyper 3.7 NT Software User's Manual
GeneScan Reference Guide

Examination of Data in GeneScan

1. Launch the GeneScan software. For evaluation of Identifiler STR data, use Mac version 3.2 or NT version 3.7 to enable analysis of five-dye electropherograms. *All explanations will be given for the Windows version of GeneScan.*
2. Under *File:New:Create New Matrix*, make a new 5-dye matrix by selecting files for matrix samples run with individual dye colors (see Applied Biosystems 2001b, pages 3-42 to 3-45). Once files have been loaded, click the "ok" button. A set of numbers, 5 rows by 5 columns, will be generated with 1.0000 diagonally from the top left to bottom right.

3. Under *File:New:Create New Size Standard*, select an appropriate sample file and define size standard peaks by highlighting peaks and typing in the known size for that peak.
4. Under *File:New:Create New Analysis Parameters*, define analysis parameters as determined from validation studies (Moretti et al., 2001), such as a peak detection threshold of 50 or 100 RFUs. The size calling method can typically be left on the default of Local Southern Method.
5. Import STR data generated following Basic Protocol 2 into a new project folder. Click on *File:New:Create Project*. Then under *Project:Add Sample Files...*, add samples from the desired run folders. Load the newly created size standard and analysis parameters into the columns adjacent to the sample names. Highlight the samples in the Analysis Control window and click “Analyze.” Results of processed samples can then be viewed in the Results Control window as color separated data with peaks sized against the internal standard.
6. Check allelic ladder peaks to make sure all alleles are above the peak-calling threshold. This can be done by double-clicking on the sample name and removing dye colors to evaluate each color or by viewing the dye colors separated in the Results Control window.

NOTE: Some laboratories may choose to perform extensive data review in GeneScan while others will prefer to go directly to Genotyper.

Genotyper Analysis

1. Launch Genotyper 3.7 NT software.
2. Using *File: Open* command, open a copy of an Identifiler macro.

NOTE: Macros are typically available for download from STR kit vendor websites or are supplied on disk or CD-ROM when a kit is purchased. Macintosh macros can be converted to PC compatible macros by simply typing .gta at the end of the file name.

3. Under *Edit: Set Preferences...*, make sure that under the import colors section all five dye colors (blue, green, yellow, red, and orange) are selected. The orange dye lane will need to be included so that the GS500 LIZ internal standard can be evaluated in Genotyper.
4. Import GeneScan processed data by clicking on *File: Import: From GeneScan File* and then locating the desired files for Genotyper processing.

5. In the macro control window, click Ctrl-1 and scroll through each sample to check that all peaks were assigned properly for the internal size standard.

NOTE: If there are any peaks that are called incorrectly, go back to GeneScan and reanalyze the sample under different analysis conditions or re-run the original sample. Correct comparisons between allelic ladder allele sizes and sample allele sizes are dependent on the internal size standard peaks being called properly.

6. After review of the internal size standard, click Ctrl-2 and execute the “Kazaam” macro. This macro compares allele sizes between the allelic ladder and each sample and makes the genotyping call for each locus. The macro should also remove stutter products.

NOTE: The Identifiler macro supplied by Applied Biosystems with their kit contains a Kazaam 20% filter (Ctrl-6) that works well for known single source samples, such as those run for DNA databasing purposes. This macro removes any peaks that are less than 20% of the highest peak in each locus size range.

7. After the Kazaam macro has successfully processed the samples, review the data by dye color and locus to ensure that peaks are correctly assigned. Edit peak calls by clicking on the label to remove it (e.g., if it is a spike or stutter peak that was improperly designated a true allele, see **Commentary**).

Note: Specific interpretation guidelines for calling STR alleles vary between human identity testing laboratories. A minimum recommended set of rules are contained in the SWGDAM STR Interpretation Guidelines (SWGDAM, 2000).

8. In the macro control window, click Ctrl-4 to create an allele table after data has been reviewed. This table can be saved and pulled into an Excel spreadsheet for further analysis. Zeros will be automatically inserted where no STR allele data are recorded.
9. Click Ctrl-5 to put allele information into a CODIS-compatible format. The CODIS-format lists sample info (name entered in Sample Sheet), category (STR locus), peak 1 (first allele), and peak 2 (second allele).

STR typing information may be plotted either with overlapping dye colors or separated. Figure 14.8.2 shows a full Identifiler DNA profile separated by dye color after processing through the Genotyper Kazaam macro.

<Insert Figure 14.8.2 full Identifiler DNA profile near here>

Alternative Protocol 1

AMPLIFICATION OF STR MARKERS: POWERPLEX[®] 16 KIT

The PowerPlex 16 kit co-amplifies the 13 CODIS STR loci, the sex-typing marker amelogenin, and two pentanucleotide repeat loci referred to as Penta D and Penta E (Krenke 2002). The pentanucleotides exhibit low stutter properties and can be useful in deciphering mixtures (see Butler 2001). The steps for sample detection (Basic Protocol 2) and analysis (Basic Protocol 3) are pretty much the same as with the Identifiler kit.

Materials

Thermal cycler (e.g., GeneAmp 9600 or 9700 in 9600-emulation mode)
PowerPlex[®] 16 STR kit (Promega Corporation, Madison, WI): includes primer mix, and PCR components such as dNTPs, MgCl₂, and buffer
AmpliTaq Gold DNA polymerase (Applied Biosystems)
0.20-mL thin-walled PCR tubes
1.5-mL sterile tube
DNA extract of appropriate biological specimens that have been previously quantified, and accompanying positive and negative controls (Unit 14.3)

PCR Setup

1. Label PCR tubes with sample names.
2. Thaw PowerPlex 16 kit Gold STaR 10X buffer and primer mix components to room temperature.

NOTE: Promega STR kit components are typically stored at -20 °C while Applied Biosystems STR kits are stored at 4 °C. Wrapping your gloved hand around the individual tubes and holding the tube for several minutes may efficiently thaw frozen components. If a precipitate is observed in the buffer tube, the solution may be heated briefly to 37 °C.

3. Vortex the thawed solutions to make sure that the contents are homogeneous.
4. For a 25- μ L PCR reaction, add 2.5 μ L Gold STaR 10X buffer, 2.5 μ L PowerPlex 16 10X Primer Pair Mix, and 0.8 μ L of AmpliTaq Gold DNA Polymerase (4 units). The remaining volume of 19.2 μ L can contain various combinations of DNA and sterile water depending on the DNA concentration.

NOTE: If more than one sample is being prepared in parallel, it is helpful to make a PCR master mix in a 1.5-mL sterile tube that can then be equally dispensed to all tubes in order to maintain consistency between samples.

5. Lightly vortex this PowerPlex 16 master mix to insure that it is homogeneous and dispense 5.8 μL to each PCR tube.
6. Add sterile water and then the extracted DNA to each PCR tube containing the PowerPlex master mix so that a total amount of 0.5-1.0 ng DNA is present.

NOTE: If the DNA sample is stored in TE, then its total volume should not exceed 20% of the reaction volume (i.e., 5 μL in a 25 μL reaction). Higher amounts of TE can adversely alter the pH of the reaction or the available magnesium concentration.

7. Setup positive and negative control samples in addition the DNA samples under testing. The positive control can be a fixed amount, such as 1.0 ng, of the 9947A kit control sample and is used to verify that PCR components are working properly. The negative control can be 2 μL of deionized water or TE buffer in place of DNA and is used to verify that PCR components are not contaminated with extraneous human DNA.
8. Cap PCR tubes.
9. Place PCR tubes into the thermal cycler.
10. Initiate a thermal cycling program that performs the following temperature steps: 95 °C for 11 min; 96 °C for 1 min; 10 cycles of 94 °C for 30 seconds, 60 °C for 30 seconds, 70 °C for 45 seconds; then 22 cycles (or down to 18 cycles if slightly higher amounts of DNA than 1 ng are used) of 90 °C for 30 seconds, 60 °C for 30 seconds, 70 °C for 45 seconds; final hold of 60 °C for 30 min and then 4 °C until samples are removed. If a GeneAmp 9700 thermal cycler is used, ensure that the temperature ramp speeds are set for 1 °C/second (9600-emulation) rather than 4 °C/second (9700-default). In addition, ramping rates of 29% from 94 °C or 90 °C down to 60 °C and 23% from 60 °C to 70 °C must be followed to ensure optimal amplification.

A PowerPlex 16 DNA profile for ~1 ng NIST SRM 2391b component 1 should look like Figure 14.8.3.

<Insert Figure 14.8.3 *PowerPlex 16 DNA profile* near here>

Alternative Protocol 2

AMPLIFICATION OF Y-STR MARKERS: Y-PLEX[®] 12 KIT

Several commercial Y-chromosome STR kits are now available. The Y-PLEX[™] 12 kit from ReliaGene Technologies, Inc. (New Orleans, LA) co-amplifies the minimal haplotype of DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and DYS385 a/b that is widely

accepted in Europe plus the two additional markers DYS438 and DYS439 recommended by the Scientific Working Group on DNA Analysis Methods (Butler 2003).

Materials

Thermal cycler (e.g., GeneAmp 9600 or 9700 in 9600-emulation mode)
Y-PLEX™ 12 kit (ReliaGene Technologies, New Orleans, LA): includes primer mix, and PCR components such as dNTPs, MgCl₂, and buffer
AmpliTaq Gold DNA polymerase, Applied Biosystems
0.20-mL thin-walled PCR tubes
1.5-mL sterile tube
DNA extract of appropriate biological specimens that have been previously quantified, and accompanying positive and negative controls (Unit 14.3)

PCR Setup

1. Label PCR tubes with sample names.
2. For each 25- μ L reaction, add 10.0 μ L 2.5X Y-PLEX 12 Primer Mix and 0.5 μ L AmpliTaq Gold DNA polymerase (5 U/ μ L).

NOTE: If more than one sample is being prepared in parallel, it is helpful to make a PCR master mix in a 1.5-mL sterile tube that can then be equally dispensed to all tubes in order to maintain consistency between samples.

3. The remaining 14.5 μ L volume can be any combination of DNA sample and sterile water to make the final DNA amount in the range of 1-2 ng.
4. Setup positive and negative control samples in addition the DNA samples under testing. The positive control can be a fixed amount, such as 1.0 ng of the male cell line 9948 or the kit control sample, and is used to verify that PCR components are working properly. The negative control can be a female DNA sample, such as the cell line 9947A. Alternatively, deionized water or TE buffer can be used in place of DNA to verify that PCR components are not contaminated with extraneous human DNA.
5. Cap PCR tubes.
6. Place PCR tubes into the thermal cycler.
7. Initiate a thermal cycling program that performs the following temperature steps: 95 °C for 10 min; 30 cycles of 94 °C for 1 min, 58 °C for 1 min, 70 °C for 1 min; final hold of 60 °C for 60 min and then 4 °C until samples are removed. If a GeneAmp 9700 thermal cycler is used, ensure that the temperature ramp speeds are set for 1 °C/second (9600-emulation) rather than 4 °C/second (9700-default).

Using ~2 ng of a human Y-chromosome DNA profiling standard (NIST SRM 2395 component A), a Y-PLEX 12 DNA profile should look like Figure 14.8.4 in a Genotyper layout.

<Insert Figure 14.8.4 *Y-PLEX 12 DNA profile* near here>

Alternative Protocol 3

SEPARATION AND DETECTION OF STR AMPLICONS: Use of Multi-capillary ABI 3100 System

The ABI Prism 3100 Genetic Analyzer is a 16-capillary array electrophoresis system that enables a higher sample throughput than the single capillary ABI 310 instrument. A batch of 96 samples can be processed in about 6 hours as opposed to about 2.5 to 3 days on the ABI 310. To track samples and control electrophoresis parameters, a user creates plate records rather than sample sheets and injection lists as described in Basic Protocol 2. Samples are injected 16 at a time from a 96-well or 384-well tray.

Another important difference between the ABI 3100 and ABI 310 is that a proper matrix must be established *before* running samples on an ABI 3100, as it is not possible to go back and re-apply a matrix later. A pre-established matrix is applied on the fly to generate color-separated data with the 16 lanes of data collected during each ABI 3100 injection. Matrix standards are different for the ABI 3100 compared to the ones used for the ABI 310. The 3100 matrix standard contains a single peak for each dye color.

Materials

Matrix standards, DS-33, for G5 filter, P/N 4323016 (Applied Biosystems)
Matrix standards, DS-32, for F filter, P/N 4323018 (Applied Biosystems)
PowerPlex Matrix Standards, 3100 (Promega Corporation), Product #DG3380
Hi-Di formamide, P/N 4311320
GeneScan-500 LIZ size standard, P/N 4322682
Size standard, GS500 ROX (for Y-PLEX 12)
Size standard, ILS 600 (for PP16), Product #DG2611 (Promega Corporation)
Centrifuge

8-channel pipet
3100 POP-4 polymer, P/N 4316355 or 3700 POP-6, P/N 4306733
ABI 3100 capillary array, 36 cm, P/N 4315931
Buffer, A.C.E. (Amresco Inc., Solon, OH)
Plate, 96-well, P/N N801-0560
Plate septum, P/N 4315933
Plate base, P/N 4317237
Plate retainer, P/N 4317241
ABI Prism 3100 Genetic Analyzer User's Manual

Instrument Setup and Matrix Definition

1. Turn on ABI 3100 instrument.
2. Turn on computer.
3. Launch 3100 Genetic Analyzer Data Collection software (version 1.0.1).
4. Put in array, buffer trays, and polymer as described in the ABI Prism 3100 Genetic Analyzer User's Manual (see pp. 3-20 to 3-25).
5. Perform spatial calibration (see pp. 4-4 to 4-13). A spatial calibration determines the position of fluorescence from each capillary onto the detector. It should be done whenever an array is changed.
6. Perform spectral calibration (see pp. 4-16 to 4-32). A spectral calibration creates a multi-component matrix to correct for overlapping of fluorescence emission spectra of the dyes detected in analysis.
 - a. Add 5 μ L of 3100 matrix standard DS-33 to 195 μ L formamide and mix.
 - b. Aliquot 10 μ L into each of 16 wells in two adjacent rows (e.g., positions A1-H1 and A2-H2).
 - c. Centrifuge plate to remove bubbles and air gap at the bottom of each well.
 - d. Under Plate View tab, click New.
 - e. Enter plate name, select Spectral Calibration for application, 96-well for plate type, and enter any additional information in the comments section to track samples.
 - f. Enter matrix name in Sample Name column next to appropriate sample positions in 96-well plate.
 - g. Select Dye Set that corresponds to the dyes being used to create the matrix. For example, with Identifiler data use G5.

- h. Select the default run module.
- i. Save the plate record.
- j. Under Plate View tab, select plate just saved from the Pending Plate Records section. Put plate in left position in autosampler. Select the “A” plate position. Select the “A” row on the Linked Plate Records section. Then click the green arrow “Run” button at the top of the screen
- k. After samples have run, review the matrix results for each capillary. If more than two or three capillaries have failed, then repeat the spectral calibration.

NOTE: It is essential that a good matrix be created before running samples in the ABI 3100 as the matrix is applied “on-the-fly” to samples as they are detected. Samples must be rerun if there is an inappropriate matrix used rather than just reanalyzed with a new matrix as can be done on the ABI 310.

Sample Preparation for Analysis

1. A 96-well plate is used instead of individual 0.5 mL tubes as described in Basic Protocol 2 for the ABI 310. Setup an appropriate number of samples tubes with an allelic ladder in addition the PCR samples amplified in Basic Protocol 1.

NOTE: Samples should be prepared in batches of 16 if possible. Formamide can be placed in any empty wells so that the inlets of all 16 capillaries are submerged during injection.

2. Mix 0.3- μ L GS500 LIZ (or 0.6- μ L GS500 ROX) size standard with 14- μ L Hi Di formamide.

NOTE: If more than one sample is being prepared in parallel, it is helpful to make a master formamide/size standard mix in a 1.5-mL sterile tube that can then be equally dispensed to all tubes in order to maintain consistency between samples. Add one extra volume for every ten samples to correct for volume lost in pipetting.

3. Add 14- μ L Hi-Di formamide/size standard mix to each well of the 96-well sample plate.

NOTE: If more than two rows (16 samples) are being prepared at the same time, it is helpful to use an 8-channel pipet, in which case the formamide/size standard mix will need to be first transferred to an open trough to enable aspiration of liquid to all tips simultaneously.

4. Carefully pipet 1- μ L of each Identifiler sample amplified in Basic Protocol 1 into each well containing the Hi-Di formamide.

NOTE: An 8-channel pipet will make sample transfer from PCR tubes or trays much easier and less prone to moving a sample into the wrong well.

5. Place a clean plate septum over the 96-well plate and gently push septum into wells.
6. Centrifuge plate for at least 10 seconds at more than 500 rpm to mix the sample, remove bubbles, and spin the liquid to the bottom of each well.
7. Inspect the sample wells after removal of the plate from the centrifuge to ensure that all liquid is in the bottom of each well. Any air gaps or bubbles can interfere with sample injection.
8. Put the septa-covered 96-well plate into the plate base and snap on the plate retainer. Make sure that the plate retainer holes are aligned with the holes in the septa.

Setup Sample Data Collection

1. Launch 3100 Genetic Analyzer Data Collection software (version 1.0.1).
2. Place the plate prepared above on the ABI 3100 instrument autosampler in either position A or B.
3. Under Plate View tab, click New.
4. Enter plate name, select GeneScan for application, 96-well for plate type, and enter any additional information in the comments section to track samples.
5. Enter sample names next to corresponding positions. Copy sample names to the Color Info column. Select Project1 for the BioLIMS Project column. Select the appropriate Dye Set: *G5* for Identifiler (from Basic Protocol 1), *D* for PowerPlex 16 (from Alternative Protocol 1), and *F* for Y-PLEX 12 (from Alternative Protocol 2). Select a Run Module (the default works well for DNA fragments up to 500 bp in size).

NOTE: Matrix names are user defined so manufacture protocols may call the PowerPlex 16 matrix Z rather than D.

6. Save the plate record.
7. Start the run as described above.

8. Data will typically be deposited following the run in a Run Folder containing 16 samples at D:\appliedbio\3100\DataExtractor\Extracted Runs\..... The individual GeneScan files can then be processed as described in Basic Protocol 3.

COMMENTARY

Background Information

The human identity testing community has focused on 22 autosomal STR loci (Table 14.8.2) and about a dozen Y-chromosome STR markers (Table 14.8.3) that are present in commercial kits.

The use of core sets of loci enables common information to be included in criminal DNA databases. The chromosomal locations, repeat motifs, allele ranges, PCR product sizes, and random match probabilities for these common autosomal STR loci are listed in Table 14.8.2.

Match probabilities will vary based on the population data used to calculate them and so the information listed in Table 14.8.2 is primarily helpful to compare the relative degree of polymorphism between the markers rather than an absolute power of discrimination.

<Insert Table 14.8.2 *common autosomal STR markers* near here >

Information on common Y-STR loci is contained in Table 14.8.3. The positions of the markers along the Y-chromosome, the repeat motif, allele range, and PCR product sizes for alleles amplified with the PowerPlex Y kit are all described. The STR diversity values for these Y-STR markers, which were calculated from 244 U.S. Caucasian males (Schoske et al., 2003), are listed to provide an indication of relative usefulness for the various markers in terms of resolving individuals with unrelated patrilineages. These Y-STRs are all linked on the non-recombining region of the Y-chromosome making them less able to differentiate two unrelated individuals and virtually impossible to distinguish fathers from sons or brothers from one another except in the case of mutation. While they are less polymorphic than autosomal STRs that are present on

different chromosomes and are shuffled with each generation, Y-STRs do have a valuable role to play in many forensic cases including sexual assaults. Male-specific primers have been created for these various loci in commercial kits such as PowerPlex Y that permit recovery of a male profile even in the presence of a large excess of female DNA.

<Insert Table 14.8.3 *common Y-chromosome STR markers* near here >

The STR markers used in human identity testing are primarily tetranucleotide repeats although several pentanucleotides (Penta D, Penta E, and DYS438) and a single trinucleotide are in use (DYS392). Tetra- and pentanucleotide markers are preferred in forensic arenas due to their lower tendency to generate stutter products that can confound interpretation of mixed DNA profiles (Butler 2001). These loci have been incorporated into a number of commercially available kits as detailed in Table 14.8.4. The power of discrimination value listed in Table 14.8.4 is a result of multiplying the random match probabilities contained in Table 14.8.2 for the appropriate loci contained in a kit. Commercial kits are preferred over in-house assays, even though the kits are more expensive, as they help simplify and standardize procedures and remove the burden of PCR component quality control from the busy end user.

<Insert Table 14.8.4 *commercial STR kits* near here >

The National Institute of Standards and Technology (NIST) provides certified values for DNA samples contained in Standard Reference Materials (SRMs). Analysis of NIST SRMs, or materials traceable to NIST, is currently required on an annual basis by FBI DNA Advisory Board Standard 9.5 for all U.S. forensic DNA laboratories (see Budowle et al., 2000). NIST SRM 2391b, PCR-based DNA Profiling Standard, is certified for the 13 CODIS STR loci as well

as the 9 additional autosomal STRs listed in Table 14.8.2. NIST SRM 2395, Human Y-chromosome DNA Profiling Standard, contains 5 male components and one female component that have been certified at 31 Y-STR loci including those described in Table 14.8.3.

Critical Parameters

Thermal cycler

In order to obtain balanced PCR product yields in multiplex STR amplifications, annealing temperatures and ramp speeds between temperatures need to be accurate and precise. Most thermal cyclers come with thermal couples that may be used to monitor the various temperatures. Thermal cyclers should be calibrated on a regular basis. Ramp speeds are important for a balanced amplification from a multiplex STR reaction. Significant deviation (e.g., 4 °C/s vs. 1 °C/s) can adversely impact balance between loci as protocols were developed by the manufacturers to work with particular thermal cycling conditions. For these reasons, rapid cyclers that might be used for field work (Belgrader et al., 1998) have not done as well with generating balanced yields of multiplexed STR systems as conventional thermal cyclers.

Quantity of DNA amplified

Using too much or too little template DNA in multiplex STR amplifications can cause problems and necessitate reanalysis in order to generate interpretable results. Too much DNA can cause split peaks due to incomplete adenylation or off-scale peaks that produce bleed-through between dye colors (Butler 2001). Too little DNA can cause loss of alleles due to stochastic PCR effects (Moretti et al., 2001). Accurate quantitation of input DNA helps to produce well balanced and on-scale STR profiles. Most commercial STR kits work best in the range of 0.5 ng to 2.5 ng.

Lab cleanliness

Keep lab area clean to prevent PCR contamination. The ABI 310 and ABI 3100 CE instrument need to also be kept clean to ensure reliable electrophoresis. Any polymer or buffer spills should be cleaned up quickly to prevent urea crystals from forming (see McCord 2003).

Data Interpretation Issues

The top part of Figure 14.8.5 illustrates some common biological artifacts often observed when evaluating STR data (see Butler 2001). First and foremost are stutter products, which arise from strand slippage during the PCR amplification process of tandem repeats. For tetranucleotide repeat loci, stutter products may be observed 4 bp shorter than the true allele and are usually less than 10% of the allele's height. Incomplete adenylation results in split peaks as shown for the locus D8S1179 in Figure 14.8.5. The “-A” peak is the full length PCR product while the “+A” peak results from the DNA polymerase producing an overhanging base. Tri-allelic patterns are most often observed in TPOX and D18S51 and probably result from a duplication of the primer binding sites on one of the two chromosomes being amplified. Variant alleles, such as the D7S820 allele 8.1 shown in Figure 14.8.5, result from an insertion or deletion of a nucleotide either within or nearby the repeat region. NIST maintains an Internet database on STR markers used in human identity testing known as STRBase (<http://www.cstl.nist.gov/biotech/strbase>). STRBase catalogues variant alleles as well as tri-allelic patterns that have been observed by forensic DNA scientists around the world.

<Insert Figure 14.8.5 *STR artifacts* near here>

The typical signal pattern for a degraded DNA sample is shown in the middle panel of Figure 14.8.5. Signal loss is observed as PCR product size increases because the number of full-length, intact molecules around a STR locus is reduced when the DNA sample has been damaged.

Moving primers closer to the repeat region to produce miniSTR systems can result in improved amplification with degraded DNA samples (Butler et al., 2003).

Samples containing biological mixtures from two or more individuals can be observed in forensic casework, especially with sexual assault evidence. Mixtures with DNA in near equal proportions can be fairly easily discerned by the presence of more than two alleles at multiple loci as shown in the bottom panel of Figure 14.8.5. Deciphering the full STR profiles of all donors can be challenging and is often performed with the aid of a known profile such as the victim's.

Anticipated Results

The availability of commercial STR kits (see Table 14.8.4) enables reliable PCR amplification of STR markers. Reagents for standard reaction volumes of 50 μ L or 25 μ L can cost a user about \$25-30 per sample. Several groups have demonstrated that it is possible to routinely obtain reliable STR results with small volume PCR thereby reducing the cost of these kit reagents. The Royal Canadian Mounted Police (LeClair et al., 2003) and Louisiana Crime Lab (Gaines et al., 2002) have published 5- μ L PCR protocols with commercial STR kits.

Time Considerations

STR samples can be typed now in a matter of hours with most of the time being spent in PCR thermal cycling. STRs have been typed in less than one hour using rapid cycling methods (Belgrader et al., 1998). With an ABI 3100, one individual can easily generate data from more than one hundred extracted DNA samples in a single day.

Looking Towards the Future of DNA Typing

The widespread use of core STR loci for national DNA databases means that these markers will be entrenched in forensic laboratories for the near and medium term (NIJ 2000). Single

nucleotide polymorphisms or SNPs are being considered for future use in forensic applications (Gill 2001) but conversion of technology and marker systems becomes increasingly difficult when millions of DNA samples would have to be re-typed with new methods in order to make the databases back compatible (Gill 2002).

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Thorough description of STR typing process with illustrated examples.

INTERNET RESOURCES

STRBase: <http://www.cstl.nist.gov/biotech/strbase/>

Website for detailed information on STR markers used in human identity testing maintained by the author at NIST. A fairly comprehensive list of publications dealing with STRs and human identity testing is included.

Y-STR Haplotype Reference Database: <http://www.ystr.org>

Website containing information on core Y-STR markers and haplotype frequencies observed in European, U.S., and Asian populations

Table 14.8.1 PCR amplification conditions for the Identifiler kit (Applied Biosystems)

Step in Protocol	Temperature and Time
Initial Incubation	95 °C for 11 minutes
Thermal Cycling	<u>28 cycles</u>
Denature	94 °C for 1 minute
Anneal	59 °C for 1 minute
Extend	72 °C for 1 minute
Final Extension	60 °C for 60 minutes
Final Soak	25 °C (Until tubes removed)

Table 14.8.2 Characteristics of common autosomal STR markers

STR Marker	Chromosomal Location ^φ	Repeat Motif	Allele Range [#]	PCR Product Size (kit)	RMP*
CSF1PO	5q33.1	TAGA	6-15	305-342 bp (Identifiler)	0.112
FGA	4q31.3	CTTT	17-51.2	215-355 bp (Identifiler)	0.036
TH01	11p15.5	TCAT	4-13.3	163-202 bp (Identifiler)	0.081
TPOX	2p25.3	GAAT	6-13	222-250 bp (Identifiler)	0.195
VWA	12p13.31	[TCTG] [TCTA]	11-24	155-207 bp (Identifiler)	0.062
D3S1358	3p21.31	[TCTG] [TCTA]	12-19	112-140 bp (Identifiler)	0.075
D5S818	5q23.2	AGAT	7-16	134-172 bp (Identifiler)	0.158
D7S820	7q21.11	GATA	6-15	255-291 bp (Identifiler)	0.065
D8S1179	8q24.13	[TCTA] [TCTG]	8-19	123-170 bp (Identifiler)	0.067
D13S317	13q31.1	TATC	8-15	217-245 bp (Identifiler)	0.085
D16S539	16q24.1	GATA	5-15	252-292 bp (Identifiler)	0.089
D18S51	18q21.33	AGAA	7-27	262-345 bp (Identifiler)	0.028
D21S11	21q21.1	[TCTA] [TCTG]	24-38	185-239 bp (Identifiler)	0.039
D2S1338	2q35	[TGCC] [TTCC]	15-28	307-359 bp (Identifiler)	0.027
D19S433	19q12	AAGG	9-17.2	102-135 bp (Identifiler)	0.087
Penta D	21q22.3	AAAGA	2.2-17	376-449 bp (PP16)	0.059
Penta E	15q26.2	AAAGA	5-24	379-474 bp (PP16)	0.030
SE33	6q15	AAAG	4.2-37	198-325 bp (SEfiler)	0.020
F13A1	6p25.1	AAAG	4-16	283-331 bp (FFFL)	0.098
F13B	1q31.3	AAAT	6-11	169-189 bp (FFFL)	0.144
FES/FPS	15q26.1	AAAT	7-14	222-250 bp (FFFL)	0.172
LPL	8p21.3	AAAT	7-14	105-133 bp (FFFL)	0.155
Amelogenin (sex-typing)	Xp22.22 Yp11.2	<i>Not applicable</i>		X = 107 bp Y = 113 bp	

^φChromosomal locations have been updated using the UCSC Human Genome Browser and the July 2003 reference sequence (<http://genome.ucsc.edu/cgi-bin/hgGateway>).

[#]Ranges are calculated from kit allelic ladders and do not represent the full range of alleles observed in world populations. (See Butler 2001 for a more complete allele listing of CODIS loci)

*RMP: random match probability for Caucasian individuals. The 13 CODIS loci data are from FBI data reported in NIJ's *The Future of Forensic DNA Testing* (2000). The product of these random match probabilities is 1.7×10^{-15} . D2S1138 and D19S433 RMP data are from the Identifiler Kit User's Manual from Applied Biosystems while the SE33 information is from the SEfiler Kit User's Manual. The Penta D, Penta E, F13A1, F13B, FES/FPS, and LPL information comes from PowerPlex 16 (PP16) or FFFL population data available from Promega Corporation.

Table 14.8.3 Characteristics of common Y-chromosome STR markers

STR Marker	Position (Mb) ^φ	Repeat Motif	Allele Range [#]	PCR Product Size (Kit)	STR diversity*
DYS19	9.44	TAGA	10-19	232-268 bp (PPY)	0.498
DYS385 a/b	20.00, 20.04	GAAA	7-25	243-315 bp (PPY)	0.838
DYS389I/II	13.92	TCTG TCTA	10-15 / 24-34	148-168 bp / 256-296 bp (PPY)	0.538/ 0.675
DYS390	16.52	TCTA TCTG	18-27	191-227 bp (PPY)	0.701
DYS391	13.41	TCTA	6-13	90-118 bp (PPY)	0.552
DYS392	21.78	TAT	7-18	294-327 bp (PPY)	0.596
DYS393	3.04	AGAT	8-16	104-136 bp (PPY)	0.363
DYS437	13.78	TCTA	13-17	183-199 bp (PPY)	0.583
DYS438	14.25	TTTTTC	8-12	101-121 bp (PPY)	0.594
DYS439	13.83	AGAT	8-15	203-231 bp (PPY)	0.639

^φPositions determined along human genome reference sequence in Jan 2003 using BLAT (see Butler 2003)

[#]Ranges are calculated from kit allelic ladders and do not represent the full range of alleles observed in world populations.

*Calculated from 244 Caucasian males (Schoske et al., 2003)

Table 14.8.4 Commercial kits for autosomal and Y-chromosome STR markers

Kit Name	Source	Loci Included	Power of Discrimination*
PowerPlex 1.1	Promega	CSF1PO, TH01, TPOX, VWA, D5S818, D7S820, D13S317, D16S539	$1:1.2 \times 10^8$
PowerPlex 2.1	Promega	FGA, TH01, TPOX, VWA, D3S1358, D8S1179, D18S51, D21S11, Penta E	$1:1.7 \times 10^{11}$
PowerPlex 16	Promega	CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, amel, D16S539, D18S51, D21S11, Penta D, Penta E	$1:3.4 \times 10^{17}$
PowerPlex ES	Promega	FGA, TH01, VWA, D3S1358, D8S1179, D18S51, D21S11, SE33, amel	$1:5.0 \times 10^{10}$
Profiler Plus	Applied Biosystems	FGA, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11, amel	$1:9.4 \times 10^{10}$
COfiler	Applied Biosystems	CSF1PO, TH01, TPOX, D3S1358, D7S820, D16S539, amel	$1:1.3 \times 10^6$
SGM Plus	Applied Biosystems	FGA, TH01, VWA, D3S1358, D8S1179, D16S539, D18S51, D21S11, D2S1338, D19S433, amel	$1:4.8 \times 10^{12}$
Profiler	Applied Biosystems	CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D13S317, amel	$1:3.9 \times 10^9$
Identifiler	Applied Biosystems	CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, D2S1338, D19S433, amel	$1:2.5 \times 10^{17}$
SEfiler	Applied Biosystems	FGA, TH01, VWA, D3S1358, D8S1179, D16S539, D18S51, D21S11, D2S1338, D19S433, SE33, amel	$1:2.4 \times 10^{14}$
CTT	Promega	CSF1PO, TPOX, TH01 (silver stain)	1:570
CTTv	Promega	CSF1PO, TPOX, TH01, VWA (silver stain or fluorescence)	1:9100
GammaSTR	Promega	D5S818, D7S820, D13S317, D16S539 (silver stain or fluorescence)	$1:1.3 \times 10^4$
FFFL	Promega	F13A1, FES/FPS, F13B, LPL (silver stain or fluorescence)	1:2700
Y-STR Kits			
Y-PLEX 6	ReliaGene	DYS19, DYS385a/b, DYS389II, DYS390, DYS391, DYS393	
Y-PLEX 5	ReliaGene	DYS389I/II, DYS392, DYS438, DYS439	
Y-PLEX 12	ReliaGene	DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439, amel	
PowerPlex Y	Promega	DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439	

*Calculated from combining random match probabilities in Table 14.8.2 of kit loci

FIGURE CAPTIONS

Figure 14.8.1 Summary of process used for forensic DNA typing with STR markers

Figure 14.8.2 Genotyper color-separated Identifiler kit result with 1 ng of NIST SRM 2391b component 1 following Basic Protocol 1. Sample detected on ABI 310 using Basic Protocol 2.

Figure 14.8.3 Genotyper color-separated PowerPlex 16 kit result with 1ng of NIST SRM 2391b component 1 following Alternative Protocol 1. Sample detected on ABI 310 using Basic Protocol 2.

Figure 14.8.4 Genotyper color-separated Y-PLEX 12 kit result with 2 ng of NIST SRM 2395 component A following Alternative Protocol 2. Sample detected on ABI 3100 using Alternative Protocol 3. The red channel containing the GS500 ROX size standard is not shown.

Figure 14.8.5 *Top panel:* Examples of common biological artifacts seen in STR typing including stutter products, incomplete adenylation (split peaks), tri-allelic patterns, and variant alleles.

Middle panel: Genotyper plot of PowerPlex 16 green loci observed in analysis of a partially degraded DNA sample from an aged blood stain. Note the signal loss with larger PCR product sizes.

Bottom panel: Genotyper plot of Identifiler green loci observed in analysis of a mixed DNA sample. Note the presence of more than two alleles at multiple loci.

Figure 14.8.1

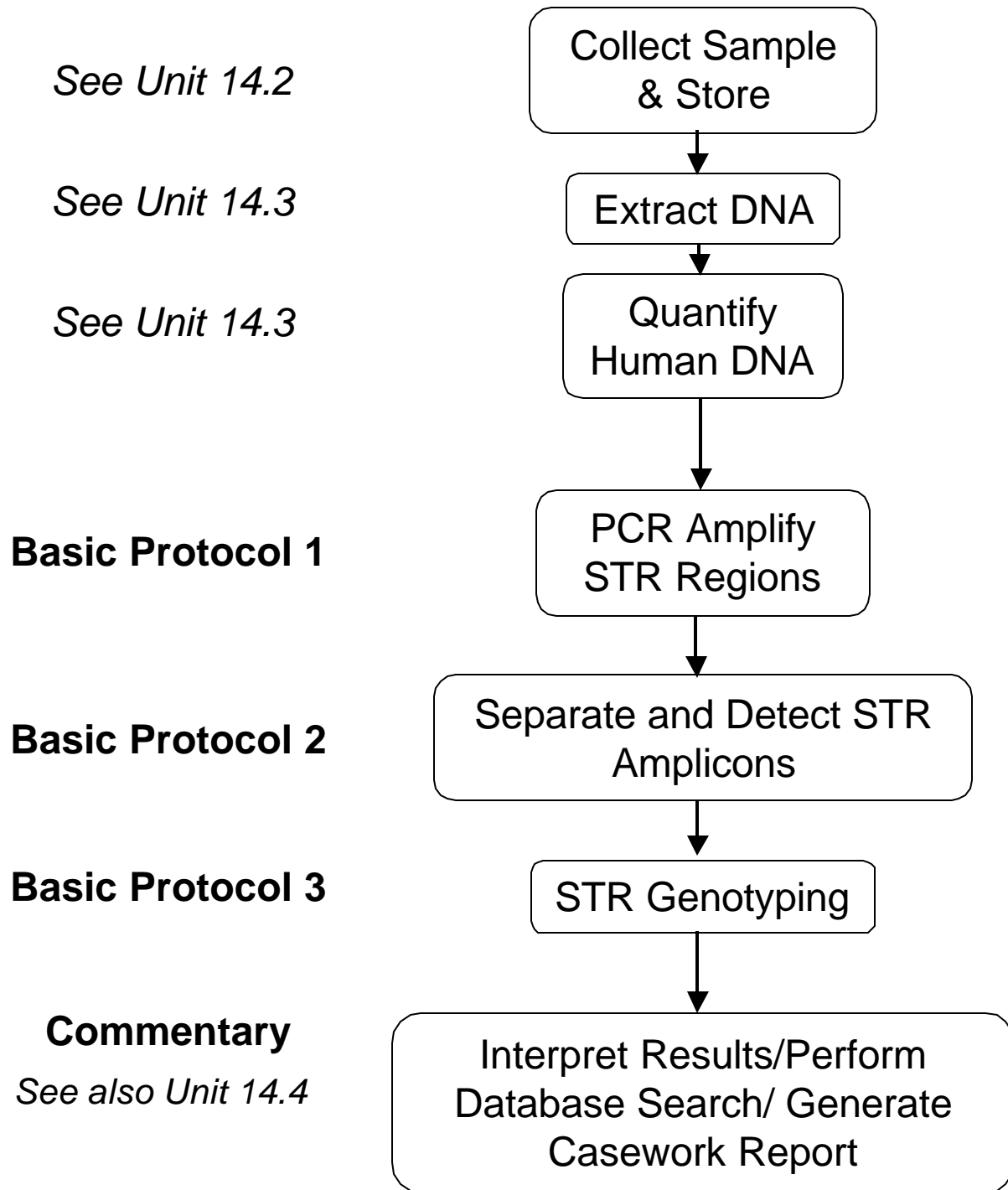


Figure 14.8.2

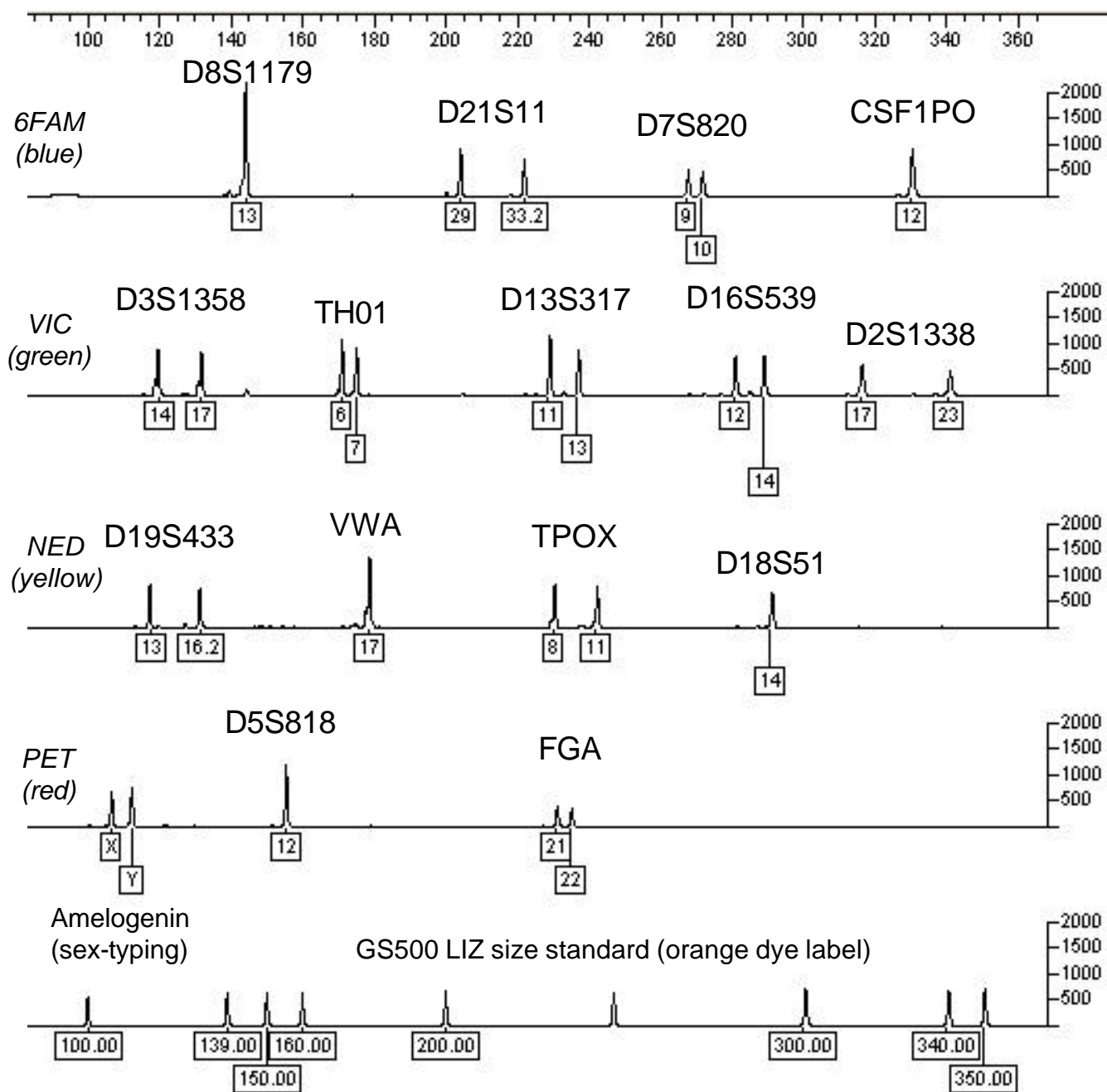


Figure 14.8.3

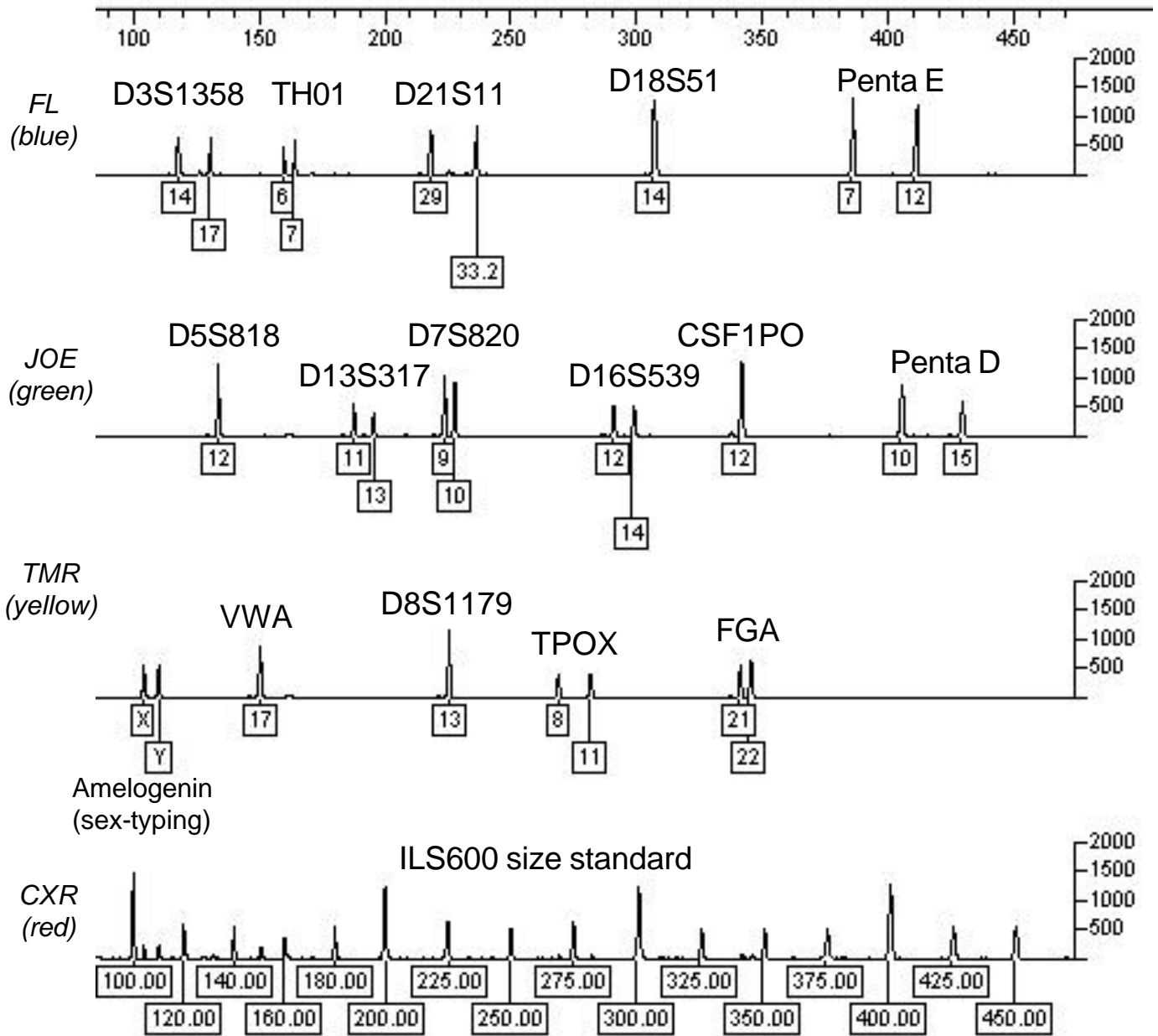


Figure 14.8.4

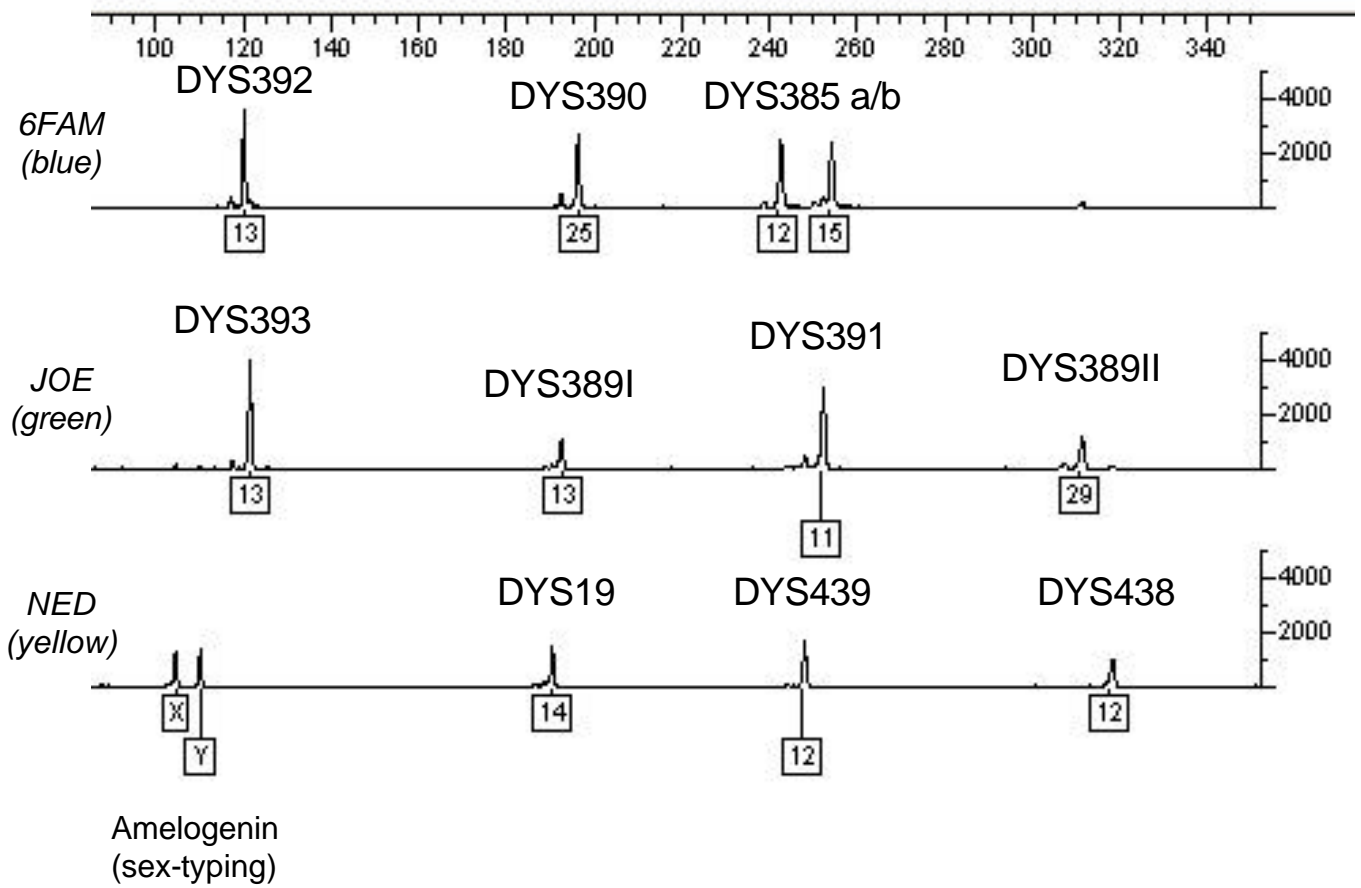
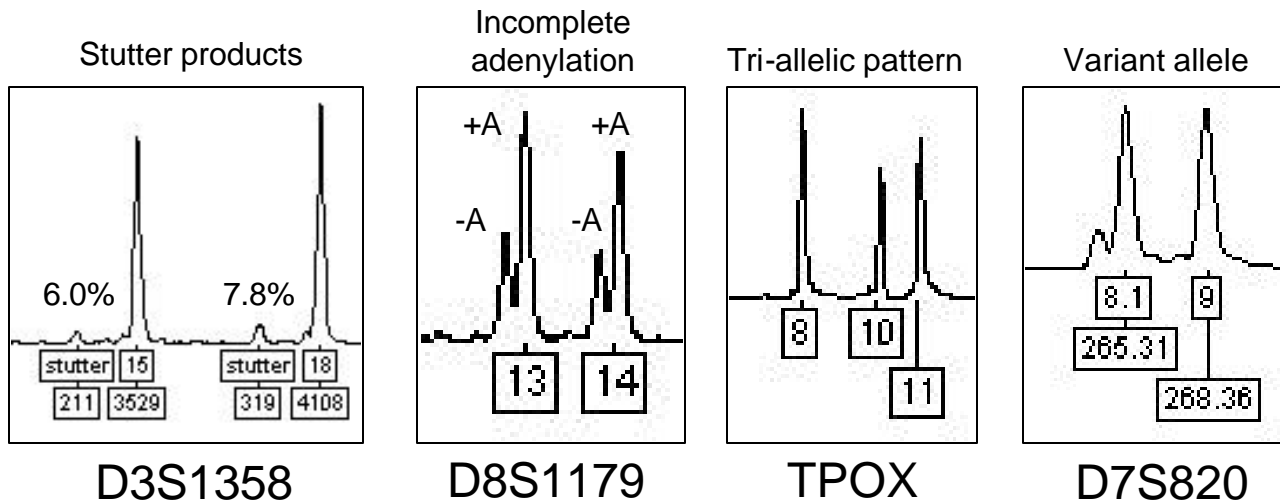
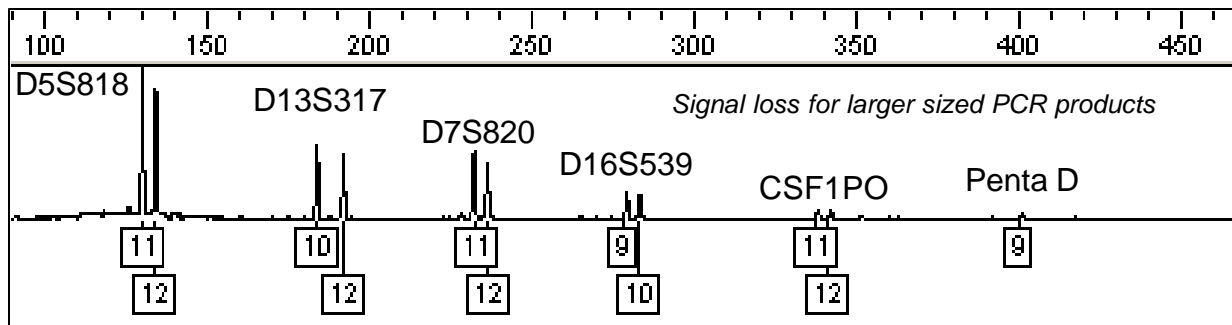


Figure 14.8.5



DEGRADED DNA

PowerPlex 16 green loci



MIXTURE

Identifiler green loci

