STR ANALYSIS AND INTERPRETATION OF PROFILER PLUS AND COFILER

PRINCIPLE:

This procedure serves as a general guideline for the interpretation of STR profiles when using the Applied Biosystems AmpFlSTR Profiler Plus and AmpFlSTR COfiler PCR Amplification and Typing Kits. These PCR-dependent kits use Short Tandem Repeat (STR) typing technology that detects length polymorphisms. This is not an exhaustive list of all casework scenarios, and the experience and discretion of the DNA Analyst are always taken into account when reporting STR profiles. A second, qualified DNA analyst will technically review all data interpretations and allele calls. If the analyst and the technical reviewer cannot agree on an allele call or data interpretation, the technical leader will be conferred with to help make a final call.

A DNA profile consists of thirteen separate locations or "loci" throughout the genome being copied or "amplified" utilizing the Polymerase Chain Reaction (PCR). These thirteen "core" loci plus a fourteenth locus to differentiate between male and female DNA are simultaneously amplified in two multiplex reactions (see below). Fluorescent tags in three different colors or "spectra" are attached to various PCR primers and enable detection of the amplified fragments or "PCR product" on the Genetic Analyzers. The PCR product is combined with an internal lane size (ILS) standard labeled with a fourth color before capillary electrophoresis (Figure 1). The PCR products are detected based on color and the time it takes the PCR product to reach the detector. The base pair sizes of the fragments are determined by interpolating between the known sizes of the ILS standard. Bins of representative alleles are created based on allelic ladders that are run with the set of samples, and when Profiler Plus and COfiler kits are combined, the thirteen "CODIS core STR loci" are amplified and detected, with two overlapping loci (D3S1358 and D7S820) appearing in both sets:

- Profiler Plus (Figure 2) detects the gender marker Amelogenin and the following nine STR loci: D3S1358, FGA, vWA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820.
- COfiler (Figure 3) detects the gender marker Amelogenin and the following six STR loci: D3S1358, D16S539, TH01, TPOX, CSF1PO and D7S820.

Either the GeneScan and Genotyper software applications (Macintosh computer) or the GeneMapperID software application (PC computer) can be used to analyze the data collected on the Genetic Analyzers. Analyzed runs are printed and any edits or notes are made directly on the printout (only permitted exception is Convicted Offenders to be exported as a CMF file).

Lists of the loci analyzed are characterized in the table below.

LOCUS	Kit	Chromosome	True Allele Bins in ladder	No. of Peaks in ladder
Blue (5-FAM):				
D3S1358	Plus COfiler	3p	*11*, **12, 13, 14, 15,** *15.2*, **16,** *16.2*, **17,** *17.2*, **18,** *18.2*, **19,** *20*	8
vWA	Plus	12p12-pter	*10*, **11, 12, 13, 14, 15,** *15.2*, **16, 17, 18,** *18.2*, **19, 20, 21,** *22*	11
FGA	Plus	4q28	*17*, *17.2*, **18,** *18.2*, **19,** *19.2*, **20,** *20.2*, **21,** *21.2*, **22,** *22.2*, **23,** *23.2*, **24,** *24.2*, **25,** *25.2*, **26, 26.2, 27,** *27.2*, **28,** *28.2*, **29,** *29.2*, **30,** *30.2*, *31*	14
D16S539	COfiler	16	**5,** *6*, *7*, **8, 9, 10, 11, 12,** *12.2*, **13, 14, 15,** *16*	9
Green (JOE):				
Amelogenin	Plus COfiler	X: p22.1–22.3 Y: p11.2	**X, Y**	2
TH01	COfiler	11	*4*, **5,** *5.3*, **6,** *6.3*, **7,** *7.3*, **8,** *8.3*,**9, 9.3, 10,** *10.3*, *11*	7
TPOX	COfiler	2	*5*, **6, 7, 8, 9, 10, 11,12, 13,** *14*	8
CSF1PO	COfiler	5	**6, 7, 8, 9, 10,** *10.2*, **11, 12, 13, 14, 15,** *16*	10
D8S1179	Plus	8	*7*, **8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,** *20*	12
D21S11	Plus	21	*23.2*, *24*, **24.2, 25,** *25.2*, **26,** *26.2*, **27,** *27.2*, **28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36,** *36.2*, *37*, *37.2*, **38,** *38.2*, *39*	22
D18S51	Plus	18q21.3	*7*, *8*, **9,** *9.2*, **10, 10.2, 11,** *11.2*, **12,** *12.2*, **13, 13.2, 14, 14.2, 15,** *15.2*, **16,** *16.2*, **17,** *17.2*, **18,** *18.2*, **19,** *19.2*, **20,** *20.2*, **21,** *21.2*,**22,** *22.2*, **23,** *23.2*, **24, 25, 26,** *27*	21
Yellow (NED):				
D5S818	Plus	5q21–31	*6*, **7, 8, 9, 10, 11, 12, 13, 14, 15, 16,** *17*	10
D13S317	Plus	13q22–31	*7*, **8, 9, 10, 11, 12, 13, 14, 15,** *16*	8
D7S820	Plus COfiler	7q11.21–22	*5*, *5.2*, **6, 7, 8,** *8.2*, **9,** *9.2*, **10, 11, 12, 13, 14, 15,** *16*	10

'True Allele Bins' in BOLD are bins represented by actual peaks in the Allelic Ladder.
'True Allele Bins' in ITALICS are bins defined by the analysis software, but not represented by peaks in the Allelelic Ladder.

Fig. 1: Internal Lane Size (ILS) Standard ROX-500.

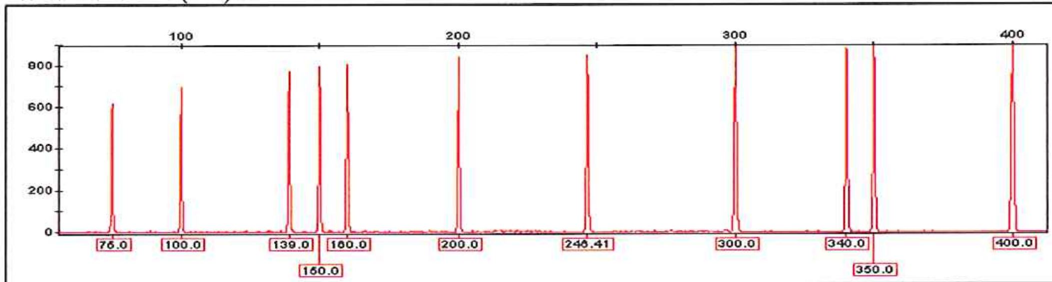


Fig. 2: Profiler Plus Ladder.

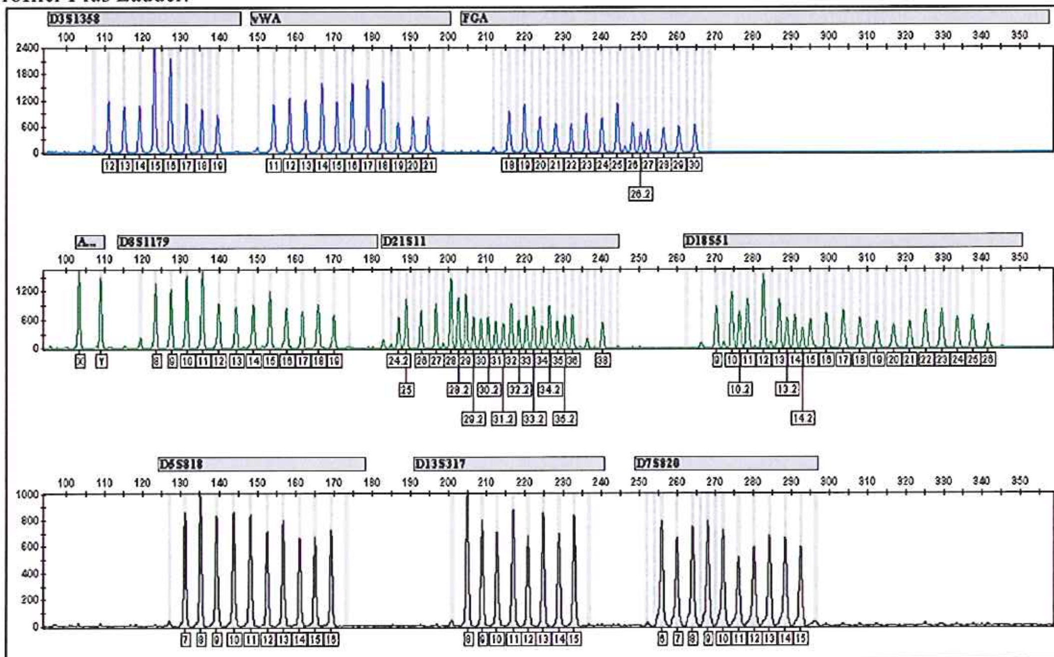
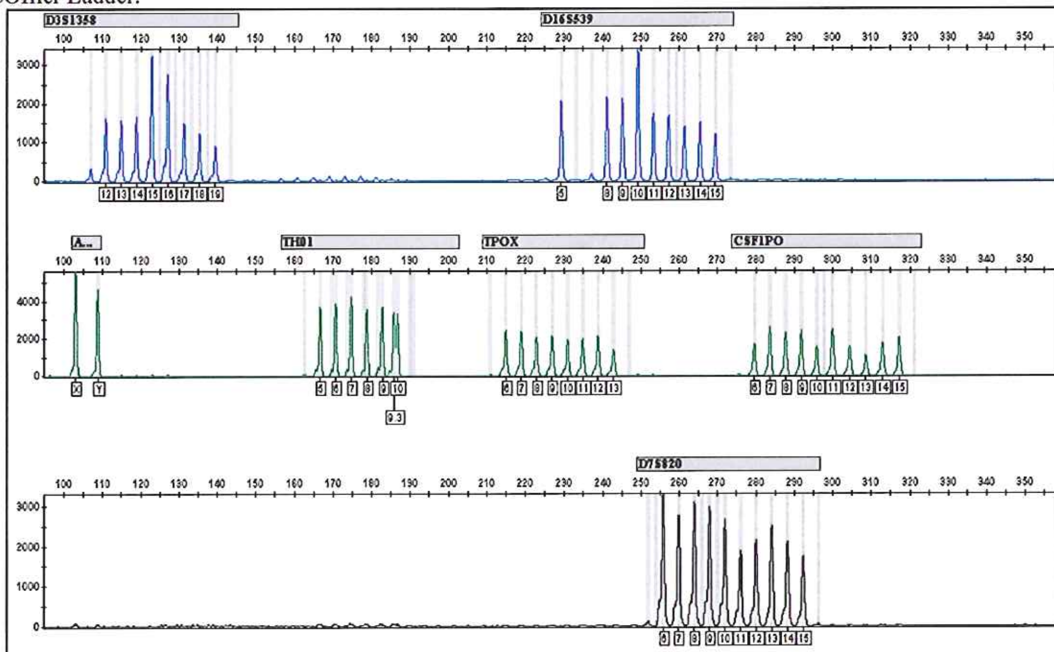


Fig. 3: COfiler Ladder.



SPECIMEN:

Samples amplified with the Applied Biosystems AmpFlSTR Profiler Plus and AmpFlSTR COfiler PCR Amplification and Typing Kits, then electrophoresed on an ABI 3130 Genetic Analyzer.

INSTRUMENTATION AND EQUIPMENT:

PC Computer

GeneMapperID v 3.2, or greater

QUALITY ASSURANCE:

1. Data must be reviewed by a proficiency-tested DNA Analyst or equivalent.
2. Edits must be made on the printed hard copies; no allele calls will be changed or deleted in the electronic format. The only exception is with convicted offenders (see protocol).
3. Any papers taken from a post-amplification room should never be taken into a pre-amplification room.

SAFETY:

1. Formamide is a potential carcinogen, and should be handled with care.
2. Prolonged computer analysis may cause muscle soreness, eye fatigue, or other discomforts. No employee shall be required to work more than two continuous hours on a video display terminal (VDT). If greater than two hours are required, then the employee shall perform other work for thirty minutes after each two-hour period on the VDT.

DNA PROFILE PARAMETERS:

1. **Nomenclature.**
   - Alleles are designated by a number, which presumably corresponds to the number of tandemly repeated segments within that allele. An individual with a D3S1358-(14,16) profile possesses one D3S1358 allele with 14 repeats and a second allele with 16 repeats.
   - Common variants exist in the Profiler Plus and COfiler systems. The most common variant is a TH01 9.3 allele. In this case one base pair deletion is present in the 10th repeat. The loci that have variant allele bins are D3S1358, vWA, FGA, D21S11, D18S51, D7S820, D16S539, TH01, and CSF1PO.
2. **Analytical threshold.**
   - The minimum peak threshold at which peaks can be reliably and reproducibly interpreted is empirically determined during the validation of the DNA profiling system on a specific platform. The average "baseline noise" in no-template controls (reagent blanks and negative controls) plus three standard deviations is tripled to determine the peak threshold.
   - The threshold is 75 rfu for the 3130 (set in GeneMapperID). Typically only alleles equal to or greater than those thresholds will be reported. Peaks below the analytical threshold can only be reported in extenuating circumstances, for purposes of exclusions, and require the approval of the Technical Leader.

- Peaks below the minimum peak threshold can help to assess the quality of a DNA profile (degradation or mixture) or determine if samples need to be reinjected or reamplified.
- If the signal for allelic ladders is too low for interpretation, they may be reanalyzed at a lower RFU threshold for the purpose of interpretation, but this must be noted in the case file and approved by the Technical Leader.
- The internal lane size standard (ILS) detection threshold can be set lower to allow GeneMapper-ID to detect and analyze the size standard, but this must be noted in the case file and approved by the Technical Leader.

3. **Internal Lane Size standard**

- The GS-500 ILS is a premixed set of DNA fragments of specific sizes (75, 100, 139, 150, 160, 200, (~245), 300, 340, 350, and 400 bp) labeled with red or ROX. The 139, 150 and 160 bp peaks appear as a tight group of three peaks and can serve as a point of reference.
- The peak of approximately 245 bp is not sized to a preset value; rather, it is used as an indicator of run precision. The range of base pair sizes for this peak will fluctuate over the course of a set of injections and is at least partly dependant on the temperature within the laboratory. The range of base pair sizes for the 245 peaks must be within 1.0 bp. If samples have 245 peaks outside of this range, it's necessary to rerun in order to call alleles in that sample.

4. **True Allele Peaks**

- A true allele typically has a peak height between the minimum peak threshold and 8100 RFU in the raw data, a fragment size that falls within the allele calling range, and has the appropriate dye color for a specific locus. However, all peaks in this range are not automatically typed as alleles. Stutter peaks, pull-up peaks, and background noise can fall within this range and will be interpreted as artifacts. Peaks less than the minimum peak threshold can be evaluated and reported only after careful analysis, but often will be deemed of little value for comparison purposes. For these reasons, the scientist's experience is the most important factor in reporting alleles, sometimes after conferring with other qualified DNA Analysts and/or the DNA Technical Leader. However, the DNA Analyst that signs the case report must feel confident in the final interpretation of any and all data pertinent to that case.
- If too much DNA is added to the PCR reaction, the fluorescence intensity from the PCR products may exceed the linear dynamic range for detection on the instrument. Samples with off-scale peaks can exhibit raised baseline, excessive pull-up in multiple spectra, and/or high stutter peaks. The peaks may even appear to have "flat tops" instead of sharp peaks. Samples with off-scale data may be diluted and rerun, reinjected for shorter times, or re-amplified with less DNA.
- Typically, within each locus, a peak balance of greater than or equal to 70% between two peaks serves as an indicator of high quality, single source DNA producing a strong signal. A peak balance between 50% and 70% should be interpreted with caution, as this could be indicative of degradation, the presence of a mixture, or low signal. Profiles that

demonstrate a reproducible imbalance of heterozygous alleles have been documented, presumably due to primer site variations or somatic mosaic.

5. **Off-Ladder Alleles**

- Off-ladder alleles are reproducible Short Tandem Repeats that do not fall within a true allele bin. Rare alleles may contain 4 bp repeat units (falling above or below the range of an allelic ladder) or repeat units less than 4 bp (falling between the bins of an the allelic ladder). If a rare allele is encountered, adding or subtracting the appropriate repeat length (1 bp if within the ladder, 4 bp if outside the ladder) from the closest allele in the ladder to create a theoretical bin (plus or minus 0.5 bp of the interpolated or extrapolated allele size).
- Off-ladder alleles must be amplified twice to document reproducibility before reporting. In order to declare a match, the same rare allele must be observed in both the questioned sample and the relevant known sample.
- Other reports of off-ladder alleles may be found on the Variant Allele Reports (www.cstl.nist.gov/biotech/strbase/var_tab.htm) and STR Fact Sheets (www.cstl.nist.gov/biotech/strbase/str_fact.htm) listed on the National Institute of Standards and Technology (NIST) Short Tandem Repeat DNA Internet Database web site (www.cstl.nist.gov/biotech/strbase/index.htm).
- Statistics for loci that have off-ladder alleles can be considered very rare, and the minimum allele frequency for that particular locus will be used as the allele frequency in calculating the random match probability for that genotype (5/2n).

6. **Stutter peaks ("n – 4" and "n + 4")**

- During the Polymerase Chain Reaction (PCR), a known phenomenon called "enzyme slippage" may occur which typically adds one fewer repeat length to a fraction of the amplified product. The most common stutter peaks have a fragment length four base pairs shorter than the true allele ("n – 4") and appear as a short peak to the left of the true allele.
- The typical maximum peak height of stutter peaks, or "stutter thresholds", are empirically determined by measuring the n – 4 peak heights at each locus for numerous samples. If a peak in the n – 4 position is less than the stutter threshold for that locus, the peak is not assigned an allele call by the analysis software. However, if a peak in the n – 4 position is greater than the stutter threshold, it may be due to off-scale signal or could indicate a mixture (see below). The sample may be reinjected for less time (2 seconds as opposed to 5 seconds) or reamplified with less DNA to aid interpretation.
- N + 4 peaks are rare, but do occasionally occur, typically in samples with too much DNA. They have a fragment length four base pairs longer than the true allele ("n + 4") and appear as a short peak to the right of the true allele. When evaluating an n + 4 peak the possibility of a mixed sample should be considered. The sample may be reinjected for less time (2 seconds as opposed to 5 seconds) or reamplified with less DNA to aid interpretation.

7. **"n-1" peaks**

   AmpFlSTR kits are optimized to add an extra adenosine nucleotide ("A") to the 3′ end of the PCR product, but when the reaction fails to add this "A" nucleotide, a peak one base pair shorter than the true allele may be observed. This usually occurs because too much DNA was added to the PCR reaction and will typically be accompanied by other indications of high signal. The sample may be reinjected for less time (2 seconds as opposed to 5 seconds) or reamplified with less DNA to aid interpretation.

8. **Pull-up Peaks**

   If too much signal is detected, an overlap in the emission spectra of the dyes causes a DNA fragment to be detected as multiple peaks in multiple colors. The pull-up peak should have approximately the same fragment size and scan number as the true peak and can be demonstrated by printing all four colors with scan number or base pair size labels. This print out showing the alignment of the true peak and its pull-up peaks should be included in interpretation. Re-injecting for less time (2 seconds as opposed to 5 seconds) or re-amplifying with less DNA may aid interpretation.

9. **Spurious Peaks/Anomalies**

   - Spurious peaks are from artifacts in the electrophoretic injection or electronic noise during data collection and are not reproducible.
   - If an anomaly is in more than one color or outside of the allele calling range, interpretation can be made from the one injection.
   - If the anomaly is only present in one color <u>and</u> within the allele calling range, the sample should be reinjected to demonstrate it is not reproducible.

10. **High Baseline/Background Noise**

    - There is signal variation at the very low range of the detector that may appear as peaks with very short peak heights. True alleles should be significantly higher than this background noise to be reported.
    - Analyst's discretion should be exercised when interpreting low-level signal so it is not confused with baseline noise. Re-running and/or re-amplifying the sample may aid interpretation.

11. **Highly Degraded Samples.**

    - Highly degraded samples must be interpreted with caution: stutter peaks are generally higher than those observed in high quality DNA; there may be spurious peaks from degradation products; and there is a greater amount of unbalanced heterozygous alleles (typically, the smaller allele will be no less than 50% of the height of the larger allele).
    - Typically the largest loci are the first to demonstrate decreased peak heights or fail to amplify ("drop out") as samples become more degraded.
    - If amelogenin does not amplify, exercise caution in calling any other loci since it may be non-human DNA

12. **Inhibited Samples.**
    - Inhibited samples are extracts that contain some impurity that inhibits the polymerase enzyme activity or otherwise impacts the efficiency of the PCR reaction.
    - Inhibited samples may show unusual stutter peaks, off-ladder alleles, allele or locus drop out patterns, as well as unbalanced heterozygous alleles.
    - Inhibited samples may mimic the appearance of degraded samples, but sometimes the pattern of loci dropping out may not correlate to the size of the loci.
    - The hallmark sign of inhibition is the inability to obtain a DNA profile even though DNA has been detected in the quantification procedure.

I. GENEMAPPER-ID ANALYSIS

This section of the protocol describes how to import and analyze data from the 3130 Genetic Analyzer instruments and analyze data in the GeneMapper-ID software. GeneMapper-ID uses the internal lane size (ILS) standard to determine the basepair sizes of peaks, assigns those peaks above the threshold with allele calls. It then uses Profiler Plus and COfiler allelic ladders to create allele size categories (or bins) based on that ladder, assigns the appropriate allele label and peak height to samples based on those bins, and filter labels from stutter peaks below the predetermined threshold level..

IMPORTING AND ANALYZING 3130 SAMPLES:

1. Launch GeneMapper-ID; choose user name from the drop down list and enter password.
2. From the File menu, choose "Add sample to project". Go to My Computer, H drive, DNA, 3130 runs.
    a. Highlight an entire run or specific samples in a run.
    b. Choose "Add to list", then "Add".
    c. From the Edit menu, choose "Sort" to arrange samples according number in 'Comment' field during run (or these numbers can be entered and edited now).
3. Under "Sample type heading", choose "allelic ladder" for the ladders, "positive control" and "negative control" for the appropriate controls, and leave as "sample" for the remaining samples (if not already specified in 'Info' field during run).
4. Under "Specimen category", choose "No export" (or "Convicted Offender" for offender profiles are going to be converted to a CMF file for NDIS upload).
5. Under "Analysis method", choose "HID_Advanced_75rfu".
6. Under "Panel", choose "AmpFlSTR_Panels_v1", then either "Profiler Plus_v1" or "COfiler_v1".
7. Under "Size standard", choose "CE_F_HID_GS500".
8. Click on the green triangle to begin analysis (*shortcut: control-R*). "Save project" automatically opens. Enter project name: the case number, multiplex name, date of run and scientist's initials. Analysis will automatically continue. The program will automatically take an average of all allelic ladders to create a standard allelic ladder for each locus.

9. Highlight the entire run or the samples of interest and choose "Analysis" and "Display plots" (*shortcut: control-L*).
10. Choose "Plots Blue-Green-Yellow" to print allele calls, and "Plots Red ILS" to print the ROX-500 Internal Lane Size standards.
11. Other standard views available are "ROX overlaid by BP", "ROX overlaid by Scan", and "Plots all 4 colors".
12. To zoom in, move the cursor to the bin area where the arrow will change to a magnifying glass, and click and drag and select an area. To zoom out, choose "View" and then "Full View" (*shortcut: control-]*) or double clicking when the cursor is a magnifying glass.
13. The manner in which samples are displayed and printed can be changed by choosing "Tools / Plot Settings" (*shortcut: control-T*) and modifying the "Display Settings".
14. Allele calls should not be edited or deleted (except for CO profiles to be exported to a CMF).

PRINTING ELECTROPHEROGRAMS:

1. After choosing the appropriate view ("Plots Blue-Green-Yellow" or "Plots Red ILS") click "File / Print" (*shortcut: control-P*). The printout will be the same way as it appears on the computer screen, except labels will print for all alleles, even if "no room for labels" appears on the screen.
2. If alleles have been edited (permitted ONLY for convicted offender DNA profiles to be exported as CMF files) choose "View" and then "Allele Changes" so edits will be noted on the electropherogram printout.
3. Select the Color LaserJet printer, and click "Print".

II. INTERPRETING PROFILER PLUS AND COFILER PROFILES

NOTE: Do not perform any editing of the electropherogram or table data on the computer. All editing and note taking must be completed directly on the printed data. The only exception is for Convicted Offender data which may be edited within GeneMapper-ID and if printed should have "allele changes" selected in the print out view.

CHECKING INTERNAL LANE SIZE STANDARD:

1. Examine the red ILS to ensure every sample injected and electrophoresed correctly. Clear, sharp internal lane standards peaks should be present in all injections.
2. Examine the labels for each GS-500 peak within each sample for correct sizing (i.e. 75, 100, 139, 150, 160, 200, (~245), 300, 340, 350, and 400 bp). The 139, 150 and 160 bp peaks appear as a tight group of three peaks and can serve as a point of reference. If peaks are missing, it's necessary to rerun in order to call alleles in that sample. Extraneous peaks need to be noted and explained on the printout.
3. The smallest and the largest of the "245 base pair" peaks in a run should differ by no more than 1.0 base pair. If there are outliers (peaks outside of the 1 base pair range), those lanes need to be assessed. If there are true alleles present (that need to be sized), the sample should be reinjected. If no alleles are present (such as in a reagent blank, negative control, or sample

with insufficient DNA to obtain a profile), the sample does not need to be reinjected. If two ladders were injected at the beginning of the run, it may be advisable to delete the first ladder before analyzing since it typically migrates slower.

CHECKING CONTROLS:

4. Examine the positive amplification control (9947A) to ensure amplification has been successful for each STR locus examined. Verify that the allele calls match the expected allele calls for that locus. See "Expected Control Values" section at the end of this protocol for correct values and what may cause deviations from the expected results.
5. Examine the negative amplification control to ensure that the amplification cocktail did not contain any trace of genetic material. See "Expected Control Values" section at the end of this protocol for correct values and what may cause deviations from the expected results.
6. Examine the reagent blank to ensure that the extraction solutions did not contain any trace of genetic material. See "Expected Control Values" section at the end of this protocol for correct values and what may cause deviations from the expected results.

CALLING ALLELES:

7. Peaks greater than or equal to the minimum peak threshold are labeled with allele calls and peak heights. Typically only peaks greater than or equal to 75 RFU are sized in GeneMapperID. GeneMapperID has an option to specifically mark off-ladder alleles (and potential pull-up areas in other spectra) with colored bins.
8. Allele numbers are assigned to peaks that match the bp size of the allelic ladders (±0.5 bp) of the corresponding dye color. Peaks that fall outside the ±0.5 bp window of allele sizes or are outside of the ladder's range are labeled "OL allele" (Off-Ladder allele). If a sample allele peak is labeled "OL allele", then the sample must be reinjected to verify the result. If the off-ladder alleles are reproduced, the sample must be reamplified to confirm the allele call.
9. Peaks in the "n-4" "stutter peak position" are automatically filtered out if they are less than the stutter threshold of the corresponding main allele peak. Stutter peaks are typically four bases shorter (n - 4), but can be 4 bases longer (n + 4) than the corresponding true allele peak in rare instances. The proportion of the stutter product relative to the main allele (percent stutter) has been measured for each locus by the manufacturer and verified by our validation study.

BLUE		GREEN		YELLOW	
LOCUS DESIGNATION	STUTTER THRESHOLD	LOCUS DESIGNATION	STUTTER THRESHOLD	LOCUS DESIGNATION	STUTTER THRESHOLD
D3S1358	15	Amelogenin	3	D5S818	12
vWA	15	TH01	10	D13S317	12
FGA	15	TPOX	10	D7S820	12
D16S539	15	CSF1PO	10		
		D8S1179	12		
		D21S11	15		
		D18S51	18		

III. INTERPRETATION OF POTENTIALLY MIXED SAMPLES

When an extract has DNA from more than one person, it is called a "mixture". A mixture can be determined by the presence of more alleles than one person could possibly possess. Evidence of a mixture at only one locus is inconclusive, and will typically still be reported as a single source. The detection of a mixture at two or more loci should be reported as a mixture.

The ratio of the two or more contributors to a mixture can vary greatly, but should be consistent across all the loci in the profile, unless there are differing degrees of degradation. The pattern of peak height ratios may assist in the extrapolation of major/minor contributors and the minimum number of contributors that could be present.

Re-amplification or re- injecting samples may prove helpful by increasing the peak heights of minor contributors. Alleles that are below the cutoff threshold should still be considered in the interpretation of the mixed DNA samples even if the alleles are not reported. When trying to deduce potential contributors in a mixture, be aware of the potential for interpreting loci that could be a mixture of two homozygous alleles as a single heterozygous contributor.

RECOGNITION OF MIXTURES

1. The detection of three or more alleles at two or more loci is a mixture.
2. Peak balance less than 50% at two or more loci may indicate a mixture. Heterozygous peak balance of a single source sample is typically 70% or better; however, heterozygous peak balance for a single source may be in the range of 50-70% due to degradation and/or stochastic effect. Additional information provided by other loci may be of assistance in these cases.
3. Stutter greater than the threshold may indicate a mixture. Additional information provided by other loci is necessary to confirm the presence of a mixture.

FIVE TYPES OF MIXTURES

Mixture Type 1:

If a mixture appears to have a clearly predominant contributor within a mixed DNA profile (approximately 3:1 ratio of the major to minor components), then the random match probability for the predominant contributor's DNA profile can be reported.

If a mixture appears to have a single contributor in the minor component, a random match probability for the minor contributor's DNA profile can only be reported for the loci that have two alleles present in the minor component (i.e. no apparent overlap between the major and minor alleles).

Mixture Type 2:

If one contributor's DNA profile is from an intimate body sample (such as the female epithelial profile from a vaginal swab) then the other contributor's DNA profile may be deduced, even for overlapping alleles. A deduced DNA profile is obtained by subtracting the alleles of the known (intimate) donor from the mixed DNA profile. For overlapping alleles, the peak height addition from the known (intimate) donor can be subtracted.

A random match probability can be reported for the deduced DNA profile. Typically, random match probabilities are not given for the intimate donor since the origin is known.

Intimate body samples include but are not limited to the following: body cavity swabs, fingernail cuttings, and swabs of the skin surface.

Mixture Type 3

If there is no distinct major and/or minor contributor and the sample is not an intimate body sample, a Probability of Inclusion statistic may be stated using the STRmix program (Dr. George Carmody Carlton University, Ottawa, Ontario, Canada). Examples of this mixture type would be:

a) A biological stain from two people with approximately the same relative proportions of DNA originating from each person. In these cases where major and minor contributors cannot be reliably separated (e.g. a 50:50 ratio mixture that does not fit Type 2 above), a Probability of Inclusion can be calculated for the entire mixture.

b) A biological stain from two individuals where there is a major contributor and one minor contributor, but certain loci for the minor component do not fit the definition of a Type 1 mixture. In these cases, a Probability of Inclusion can be calculated for the minor contributor; a random match probability can be calculated for the major contributor's DNA profile.

c) A biological stain from three or more people where there is no clear major or clear minor contributor. In these cases, a Probability of Inclusion can be calculated for the entire mixed DNA profile.

d) A biological stain from three persons where there is one major contributor and two minor contributors. In these cases, a Probability of Inclusion can be calculated for the minor contributors' DNA profile; and a random match probability can be calculated for the major contributor's DNA profile.

When STRmix is employed, the following conditions must be met:

1. All alleles found in the known reference profile must be present at a given locus (i.e. no allelic drop-out) in order for the locus to be included in the Probability of Inclusion calculation.
2. A minimum of three loci showing evidence of a mixture should be entered into the STRmix program when calculating the Probability of Inclusion Statistic.
3. Mixtures that contain more than three contributors will provide very limited statistics and should be interpreted with caution (see Mixture Type 4 below).

When STRmix is employed, statements similar to the following can be made:

a) TWO PEOPLE, NO CLEAR MAJOR OR MINOR: "The mixture of DNA profiles obtained from the human bloodstain (Item 1) **is consistent with** the known samples of John Doe (Item 2) and Jane Doe (Item 3). Based on the FBI Caucasian database, it is estimated that only 1 in * people **is a potential contributor to this profile.**"

b) TWO PEOPLE, CLEAR MAJOR, NO CLEAR MINOR: "A mixture of DNA profiles was obtained from the human bloodstain (Item 1). The major contributor **matches** the DNA profile of John Doe (Item 2). The minor component **is consistent with** the DNA profile of Jane Doe (Item 3). Based on the FBI Caucasian database, it is estimated that only 1 in * people **is a potential contributor to the minor component of this profile.**"

Mixture Type 4

If a mixture appears to contain four or more contributors, the following conclusion may be used:

> "Due to the complexity of the mixture of DNA profiles obtained from the human bloodstain (Item 1), comparison to known samples would not provide strong statistical evidence."

Mixture Type 5

If a mixture appears to be uninterpretable (i.e. mixture shows excessive allelic dropout, degradation and/or preferential amplification), the following conclusion may be used:

> "Due to the limited genetic information in the mixture of DNA profiles obtained from the human bloodstain (Item 1), no meaningful comparison can be made to known samples."

EXPECTED CONTROL VALUES:

1. Positive PCR Control (DNA 9947A):
    - The profile for the Positive control should be as follows:

D3S1358 = 14, 15	**vWA** = 17, 18	**FGA** = 23, 24	**D16S539** = 11, 12
TH01 = 8, 9.3	**TPOX** = 8,8	**CSF1PO** = 10, 12	**Amelogenin** = X, X
D8S1179 = 13, 13	**D21S11** = 30, 30	**D18S51** = 15, 19	
D5S818 = 11, 11	**D13S317** = 11, 11	**D7S820** = 10, 11	

    - If the positive control does not work or does not type correctly, repeat the injection.
    - If the positive control does not work or types incorrectly repeatedly, the test results for that set of amplifications will be rendered inconclusive and need to be reamplified.
    - Possible explanations for an incorrect or failed positive control include faulty control DNA, carry-over of amplification product, contamination, failure to add control DNA or instrument failure.
2. Negative PCR Control:
    - There should be no interpretable DNA profile in the Negative PCR Control.
    - If the Negative PCR Control gives an interpretable STR profile repeatedly, the test results for that set of amplifications will be rendered inconclusive and need to be reamplified.
3. Reagent Blank Control:
    - There should be no interpretable DNA profile in the Reagent Blank Control.
    - If the Reagent Blank Control gives an interpretable STR profile, the Reagent Blank Control should be reinjected. If the Reagent Blank gives an interpretable profile repeatedly, the Reagent Blank should be re-amplified (sample permitting). If the profile

is not repeated, the results can be reported; if any interpretable profile is detected repeatedly, then the samples extracted with that particular Reagent Blank will be rendered inconclusive. If sample size permits, DNA can be freshly extracted from the pertinent test samples with a new Reagent Blank Control. The lots of reagents used should be considered potentially contaminated and QC tested as soon as possible.

IV. STATISTICAL ANALYSIS

Follow the SOP "Genetic Analysis" for Profiler Plus and COfiler to determine the statistical significance of a match.

REFERENCES:

Applied Biosystems 3130 Genetic Analyzer Getting Started Guide, Rev. B, Applied Biosystems, 2004.

Applied Biosystems 3130 Genetic Analyzer Maintenance, Trouble Shooting and Reference Guide, Rev. B, Applied Biosystems, 2004.

GeneMapper ID Software v3.1 Human Identification Analysis User Guide, Applied Biosystems, November 2003.

User Bulletin: GeneMapper ID software: Installation Procedures and New Features for GeneMapper ID Software v3.2, Applied Biosystems, 2004.

AmpFlSTR Profiler Plus™ PCR Amplification Kit User's Manual, Applied Biosystems., 2000.

AmpFlSTR COfiler™ PCR Amplification Kit User's Manual, Applied Biosystems, 2005.

Butler, J.M. Forensic DNA Typing, 1st and 2nd Editions, Elsevier Academic Press, 2001 and 2005.

Carmody, George, Interpretation and Calculations of DNA Mixtures binder, Maine State Police Crime Laboratory.

Maine State Police Crime Laboratory Profiler, Profiler Plus, COfiler Validation Folders, Forensic Biology Section.