Capillary Electrophoresis in DNA Analysis

Data Interpretation

NEAFS Workshop
Mystic, CT
September 29-30, 2004

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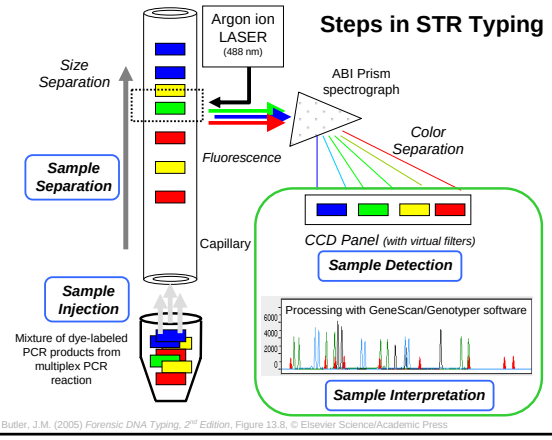
FIU Florida International University
Miami's public research university

Outline for Workshop

- Introductions
- STR Analysis
- Introduction to CE and ABI 310
- Data Interpretation
- Additional Topics – Real-time PCR and miniSTRs
- Higher Throughput Approaches
- Troubleshooting the ABI 310 (Participant Roundtable)
- Additional Topics – Y-STRs, validation, accuracy
- Review and Test

Steps in STR Typing



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 13.8, © Elsevier Science/Academic Press

NIST Instruments Used for STR Typing

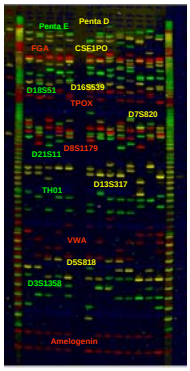
GeneAmp 9700

480 and 9600

ABI 310

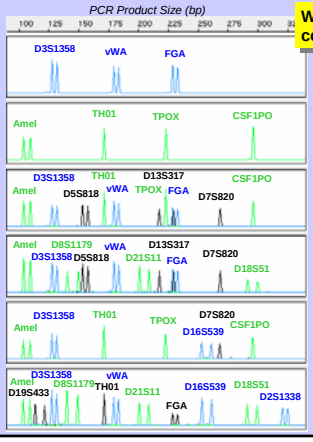
ABI 3100

FMBIO III Gel Imager System



PowerPlex 16 BIO

We work with all of the different commercial STR kits



Blue

Green I

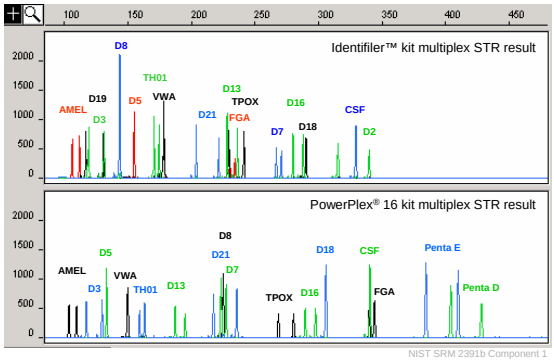
Profiler™

Profiler Plus™

COfiler™

SGM Plus™

Same DNA Sample with Two Commercial 16plex STR Typing Kits



U.S. Population Study with Identifiler STR Kit

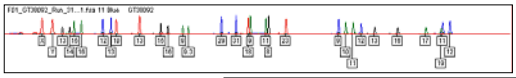
J. Forensic Sci. 48(4):908-911

FOR THE RECORD

Allele Frequencies for 15 Autosomal STR Loci on U.S. Caucasian, African American, and Hispanic Populations*

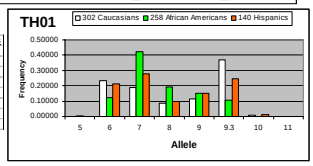
5 µL PCR protocol on ABI 3100
Reagents cost only $3.53 per sample
700 U.S. samples examined

Identifiler™



TH01 Allele Frequencies

	302 Caucasians	258 African Americans	140 Hispanics
5	0.00166	0.00388	
6	0.23179	0.12403	0.21429
7	0.19040	0.42054	0.27857
8	0.08444	0.19380	0.09643
9	0.11424	0.15116	0.15000
9.3	0.36755	0.10465	0.24643
10	0.00828	0.00194	0.01429
11	0.00166		

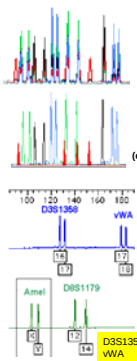


http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm

Sample Interpretation Overview

- Data collection on analytical instrument (e.g., ABI 310 or ABI 3100)
- Peak identification and sizing — *Done completely or with aid of computer program(s)*
- Correlation of peak sizes to STR allele repeat number through comparison with previously or concurrently run allelic ladder
- Review of "called" alleles with editing where needed according to laboratory-specific interpretation guidelines
- Transfer of final reviewed allele calls from STR profile to table of genotypes

Steps in STR Genotyping Process



Matrix file

Internal sizing standard (e.g., GS500-ROX)

Allelic ladder sample

Data Collection

Peak Identification

Color Separation

Peak Sizing

Comparison to Allelic Ladder

Genotype Assignment to Alleles

Data Review by Analyst/Examiner

Confirmation of Results by Second Analyst/Examiner

Data Collection software

GeneScan software

Genotyper software

Expert Systems under Development (e.g., True Allele)

D3S1358	16,17
vWA	17,18
Amel	X,Y (male)
D8S1179	12,14

Adapted from Butler (2001) Forensic DNA Typing, Figure 13.1

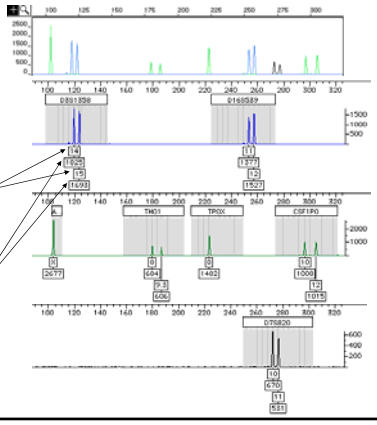
COfiler STR data

GeneScan view

Genotyper view

Allele call (repeat number) determined by comparison of peak size (bp) to allelic ladder allele peak sizes run under the same electrophoretic conditions

Peak height in relative fluorescence units (RFUs)

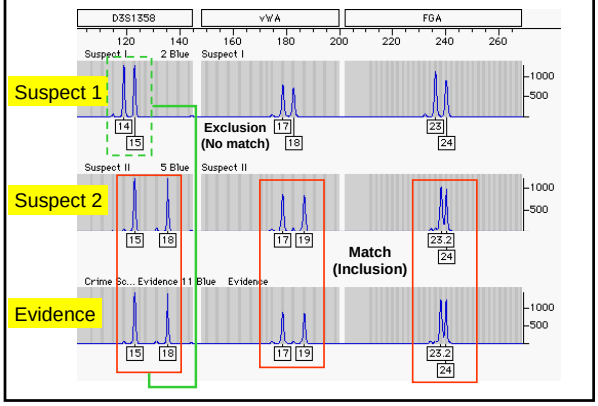


Three Possible Outcomes

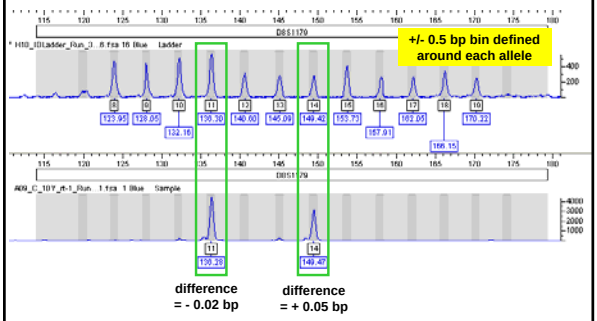
Butler, J.M. (2001) *Forensic DNA Typing*, p. 202

- **Match** – Peaks between the compared STR profiles have the same genotypes and no unexplainable differences exist between the samples. Statistical evaluation of the significance of the match is usually reported with the match report.
- **Exclusion** – The genotype comparison shows profile differences that can only be explained by the two samples originating from different sources.
- **Inconclusive** – The data does not support a conclusion as to whether the profiles match. This finding might be reported if two analysts remain in disagreement after review and discussion of the data and it is felt that insufficient information exists to support any conclusion.

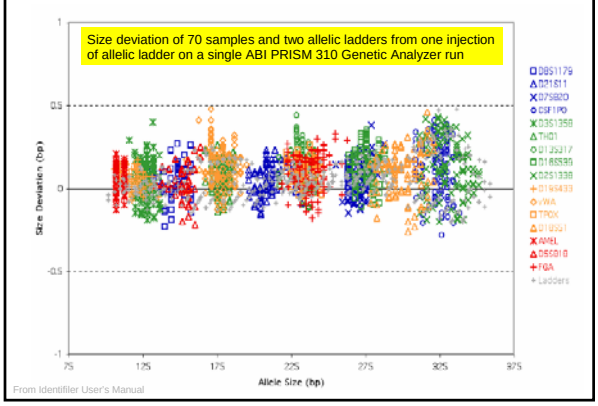
Crime Scene STR Profile Compared to Two Suspects



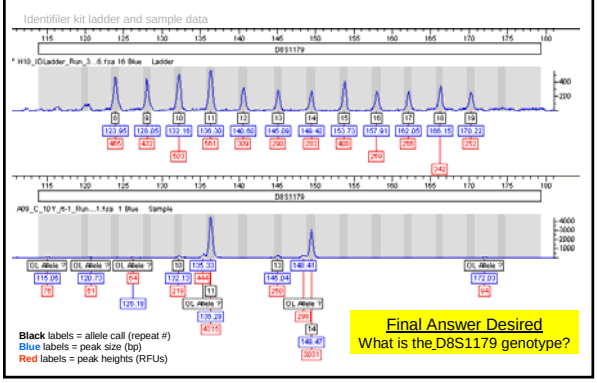
Comparison of Allelic Ladder to Samples to Convert Size into Allele Repeat Number

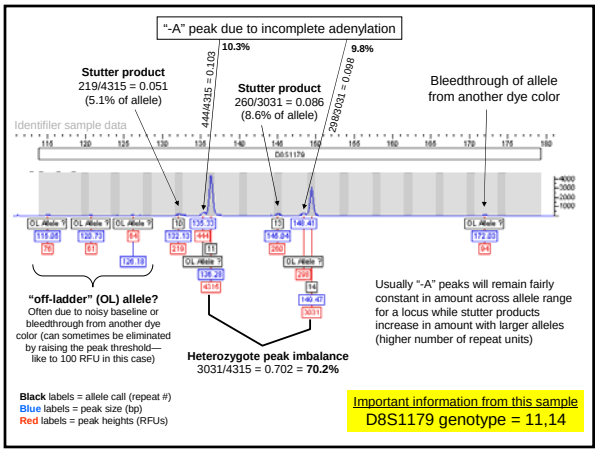


Precision from Run-to-Run on ABI 310



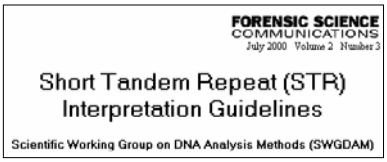
Data to be Reviewed from Electropherograms





SWGDAM STR Interpretation Guidelines

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/strig.htm



The interpretation of results in casework is a matter of professional judgment and expertise. Not every situation can or should be covered by a preset rule. It is important that the laboratory develops and implements written guidelines for interpretation of analytical results. This document provides a framework for the laboratory to develop short tandem repeat (STR) interpretation guidelines. The laboratory's interpretation guidelines should be based upon validation studies, data from the literature, instrumentation used, and/or casework experience.

SWGDAM STR Interpretation Guidelines

1. Preliminary Evaluation of Data

1.1. The laboratory should develop criteria to determine whether the results are of sufficient intensity/quality for interpretation purposes using methods appropriate for the detection platform. These criteria should be determined by evaluating data generated by the laboratory.

1.1.1. When quantitative results (e.g., peak amplitude) are used to evaluate STR profiles, the results should be examined to determine if they meet the laboratory's defined analytical and interpretational threshold(s).

1.1.1.1. The analytical threshold(s) is defined as the minimum and maximum intensity thresholds that are determined to assign alleles.

1.1.1.2. The interpretational threshold should be defined empirically.

1.1.2. When quantitative results are not used, the laboratory should establish criteria to interpret alleles based on visual inspection of gel images.

1.2. The laboratory should develop criteria to evaluate internal lane size standards and/or allelic ladders.

1.3. Controls are required to assess analytical procedures.

1.3.1. The laboratory should establish criteria for evaluation of the following controls, including but not limited to: reagent blank, amplification blank, and positive control.

1.3.2. The laboratory should develop criteria for the interpretation and documentation of results in the event that the controls do not perform as expected.

1.4. A laboratory using STR multiplexes that contain redundant loci should establish criteria regarding the concordance of such data.

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/strig.htm

SWGDAM STR Interpretation Guidelines

2. Designation

2.1. The laboratory should establish criteria to assign allele designations to appropriate peaks or bands.

2.1.1. *Locus Designation:* The laboratory should establish criteria to address locus assignment for alleles.

2.1.2. *Allele Designation:* The laboratory should designate alleles in accordance with Combined DNA Index System (CODIS) recommendations.

2.1.2.1. Whenever possible, allele designation should be based operationally on the number of repeat sequences contained within the allele and by comparison to an allelic ladder.

2.1.2.2. The designation of alleles containing an incomplete repeat motif (i.e., an off-ladder allele falling within the range spanned by the ladder alleles) should include the number of complete repeats and, separated by a decimal point, the number of base pairs in the incomplete repeat (e.g., FGA 18.2 allele).

2.1.2.3. If an allele falls above the largest or below the smallest allele of the allelic ladder, the allele should be designated as either greater than (>) or less than (<) the respective ladder allele, or when appropriate interpolation can be used.

2.2. Artifacts can occur and should be noted. These may include, but are not limited to, the following: pull-up, stutter, and nontemplate nucleotide addition. The laboratory should establish guidelines based on empirical data (obtained internally or externally) to address the interpretation of these and other artifacts.

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/strig.htm

SWGDAM STR Interpretation Guidelines

3. Interpretation of Results

3.1. The laboratory should define conditions in which the data would lead to the conclusion that the source of the DNA is either from a single person or more than one person. This may be accomplished by an examination of the number of alleles at each locus, peak height ratios, and/or band intensities.

3.1.1. *Single Contributor:* A sample may be considered to be from a single contributor when the observed number of alleles at each locus and the signal intensity ratios of alleles at a locus are consistent with a profile from a single contributor. All loci should be evaluated in making this determination.

3.1.2. *Mixtures With Major/Minor Contributors:* A sample may be considered to consist of a mixture of major and minor contributors if there is a distinct contrast in signal intensities among the alleles. The difference is evaluated on a case-by-case context. All loci should be evaluated in making this determination.

3.1.3. *Mixtures With a Known Contributor(s):* In some cases, when one of the contributors (e.g., the victim) is known, the genetic profile of the unknown contributor may be inferred. Depending on the profiles in the specific instance, this can be accomplished by subtracting the contribution of the known donor from the mixed profile.

3.1.4. *Mixtures With Indistinguishable Contributors:* When major or minor contributors cannot be distinguished because of similarity in signal intensities or the presence of shared or masked alleles, individuals may still be included or excluded as possible contributors.

3.2. The laboratory should have guidelines for interpretation of partial profiles (i.e., profiles with fewer loci than tested) that may arise from degraded or limited quantity DNA or from the presence of polymerase chain reaction (PCR) inhibitors.

3.3. The laboratory should establish guidelines to interpret profiles that exhibit potential stochastic effects (e.g., allele dropout and/or substantial imbalance of alleles).

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/strig.htm

SWGDAM STR Interpretation Guidelines

4. Conclusions

4.1. The laboratory should prepare guidelines for formulating conclusions resulting from comparisons of single source samples and mixtures with known reference samples.

4.1.1. General categories of conclusions include, but are not limited to: inclusion or match, exclusion or nonmatch, inconclusive or uninterpretable, and no results.

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/strig.htm

SWGDAM STR Interpretation Guidelines

5. Statistical Interpretation

5.1. The source of the population database(s) used should be documented. Relevant population(s) for which the frequency will be calculated should be identified.

5.2. The formulas used in calculating the frequency of a DNA profile should be defined for the following:

5.2.1. Heterozygote profiles

5.2.2. Homozygote profiles

5.2.3. Composite profiles (i.e., multiple locus profiles)

5.2.4. Minimum allele frequencies

5.2.5. Mixture calculations

5.2.6. Biological relationships, where appropriate

5.3. When used, criteria for the declaration of source attribution should be documented.

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/strig.htm

SWGDAM STR Interpretation Guidelines

6. References/Suggested Readings

Committee on DNA Forensic Science, National Research Council. *An Update: The Evaluation of Forensic DNA Evidence.* National Academy Press, Washington, DC, 1996.

DNA Advisory Board. Quality assurance standards for convicted offender DNA databasing laboratories (approved April 1999), *Forensic Science Communications* (July 2000) 2. Available at www.fbi.gov/programs/lab/fsc/backissu/july2000/codispre.htm

DNA Advisory Board. Quality assurance standards for forensic DNA testing laboratories (approved October 1998), *Forensic Science Communications* (July 2000) 2. Available at www.fbi.gov/programs/lab/fsc/backissu/july2000/codispre.htm

DNA Commission, ISFH. DNA recommendations: 1994 report concerning further recommendations regarding PCR-based polymorphisms in STR (short tandem repeat) systems, *Forensic Science International* (1994) 69:103–104.

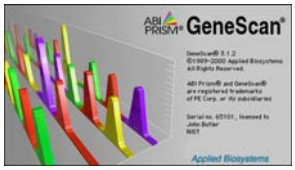
Federal Bureau of Investigation. *National DNA Index System (NDIS) Procedures Manual.* U.S. Department of Justice, Washington, DC, February 1999 (revised).

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/strig.htm

GeneScan and Genotyper Review of STR Data

- GeneScan
  - Apply matrix, size standard, and analysis parameters
  - Remove the peak designation from the "250 bp" peak in the GS500 ROX/LIZ internal size standard
  - Confirm that all alleles in the allelic ladder have been designated as peaks
- Genotyper
  - Scan sizes of all peaks in the internal size standard to confirm that they are correct (especially the 340 bp peak)
  - Run genotyping macro (Kazaam or Power)
  - Review electropherograms and edit data primarily through removing labels on peaks determined not to be alleles
  - Create a table of final allele calls for export

GeneScan® Software

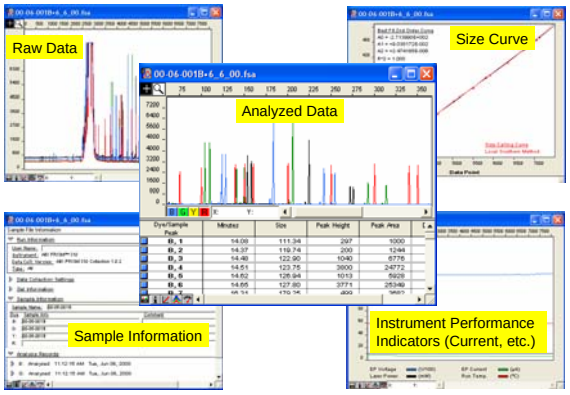


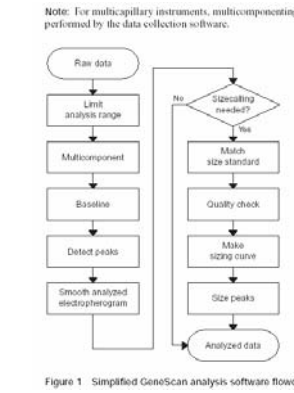
Macintosh	Windows NT
2.1	3.7 (5-dye)
3.1	3.7.1 (5-dye)
3.1.2 (5-dye)	

- Calls peaks (based on threshold values)
- Separates colors with matrix file
- Sizes peaks with internal size standard

ABI manual is P/N 4303189

GeneScan Software Screen Views

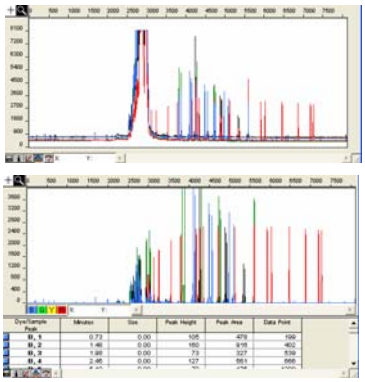




Flowchart of Steps Used by GeneScan Software to Produce Analyzed STR Data

From Applied Biosystems User Bulletin, ABI Prism GeneScan Analysis Software for the Windows NT Operating System

Baseline Subtraction

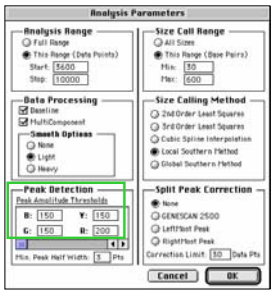


Peaks are filtered to produce normalized data

Analysis Parameters

GeneScan Analysis Parameters

Macintosh version 3.1

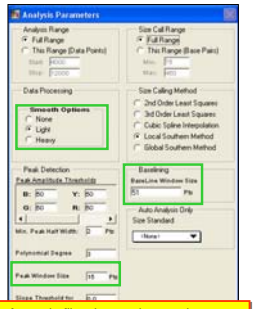


NT version 3.7

GeneScan Analysis Parameter Differences

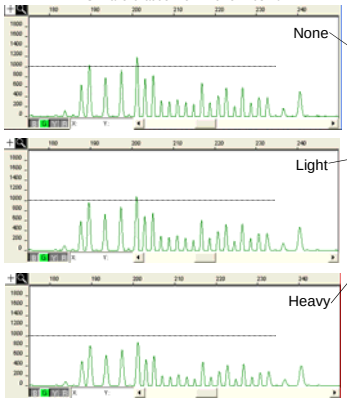
NT version 3.7 Default

NT version 3.7.1 Default

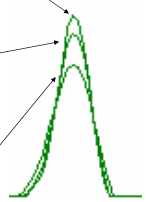


GeneScan® Analysis version 3.7.1 enables analysis of sample files that, under certain circumstances, might have failed under v3.7. Several minor improvements were implemented to increase the robustness of the sizecaller and improve the baselining function to eliminate negative peak area.

D21S11 allelic ladder from Profiler Plus kit

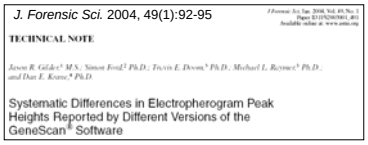


Effect of Smoothing Options



Baseline noise is reduced with smoothing but so is peak height along with resolution between closely spaced peaks (e.g., TH01 9.3/10)

Differences between Mac and NT versions of 310 Data Collection

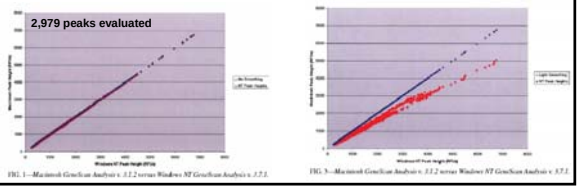


J. Forensic Sci. 2004, 49(1):92-95

TECHNICAL NOTE

Systematic Differences in Electropherogram Peak Heights Reported by Different Versions of the GeneScan® Software

The NT version of GeneScan captures peak height information prior to smoothing

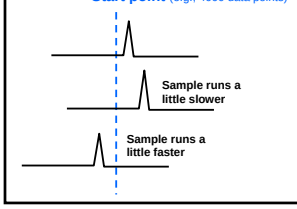


Be Careful when Limiting the Analysis Range

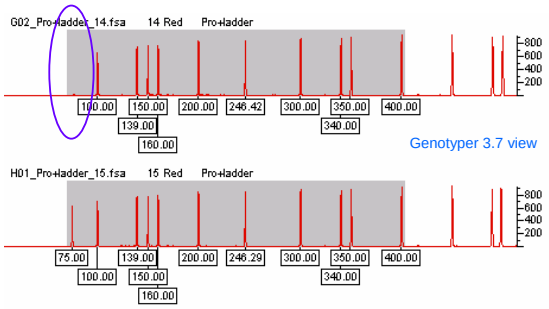
Do not put too close to peaks that are needed

There will be some variation from run to run in terms of scan numbers

Start point (e.g., 4000 data points)

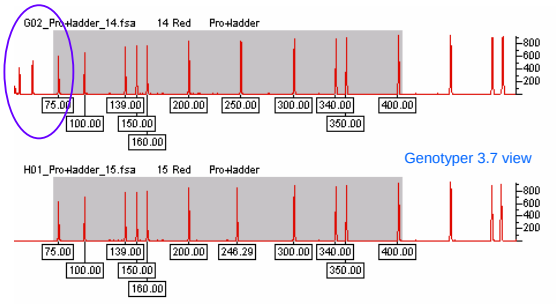


Peaks Seem to Disappear from Electropherograms if the Analysis Start Position is too Late

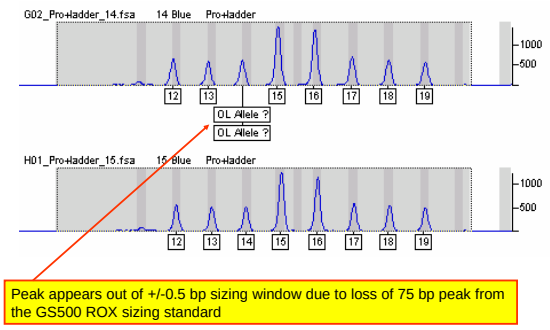


Genotyper 3.7 view

Must go back to GeneScan software and reduce Analysis start position to capture the 75 bp peak…



Appearance of "poor precision" may be due to failure to include a peak in the internal size standard for that sample

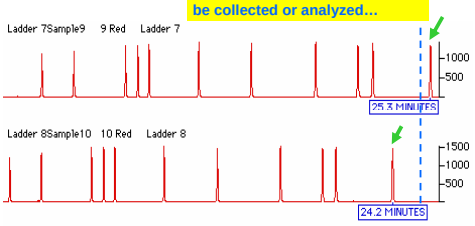


Peak appears out of +/-0.5 bp sizing window due to loss of 75 bp peak from the GS500 ROX sizing standard

Be careful about the stop point for data collection and analysis

There will be some variation from run to run in terms of scan numbers

If a stop point of 25 minutes was used, the 400 bp peak would not be collected or analyzed…

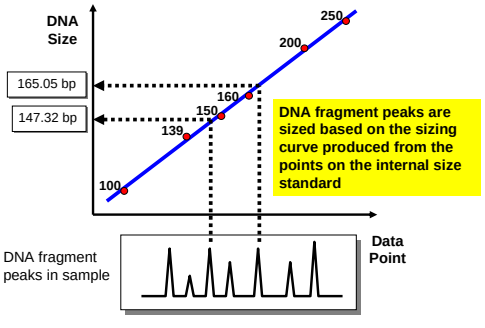


Approx. 1 minute difference in these runs

Data from Debbie Hobson (2001) FBI Laboratory

Internal Size Standards and Sizing Algorithms

Process of Sizing DNA Fragments Using an Internal Standard

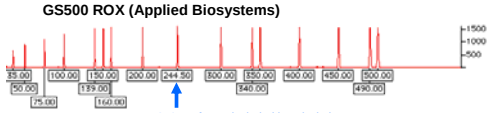


DNA fragment peaks are sized based on the sizing curve produced from the points on the internal size standard

Butler, J.M. (2001) *Forensic DNA Typing*, Figure 13.2(b), ©Academic Press

Internal Sizing Standards

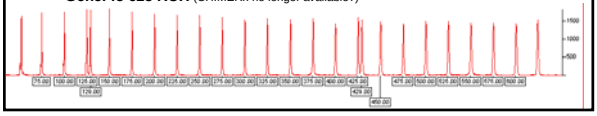
GS500 ROX (Applied Biosystems)



"**250 peak**" not included in calculations

ILS600 CXR (Promega)

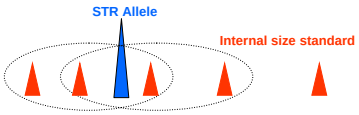
GeneFlo 625 ROX (CHIMERx-no longer available?)



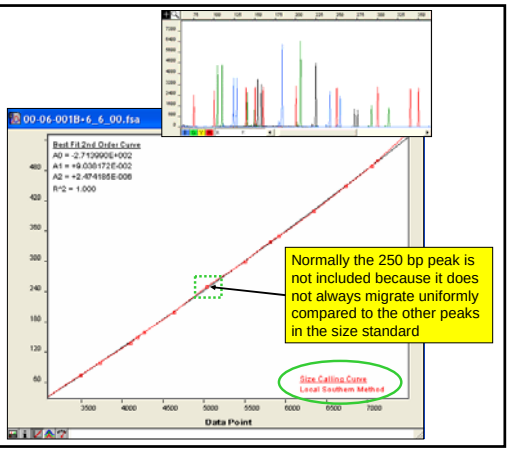
Local Southern Sizing Algorithm

- Local Southern is commonly used but may not be the best in all situations
- Local Southern involves using 2 peak above and 2 peaks below an unknown peak from the internal size standard to make a calculated DNA size

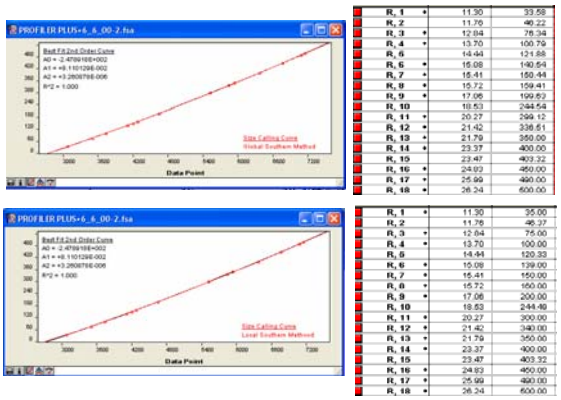
Elder, J. K. and Southern, E. M. (1983) Measurement of DNA length by gel electrophoresis II: Comparison of methods for relating mobility to fragment length, *Analytical Biochemistry* 128:227-231.



$$\frac{X_1 + X_2}{2} = \text{Size of STR Allele}$$



Global Southern vs Local Southern Methods



Global Southern Sizing

- **Global Southern Method:** Generates best-fit curve from all matched fragments in the size standard
- **Local Southern Method:** Generates best-fit curve from only nearby internal lane standard data points (usually two peaks on either side of the unknown)

There is value in Global Southern Sizing for STRs when temperature variations within a run exist:

- Hartzell, B., Graham, K., McCord,B. (2003) *Forensic Sci. Int.* 133:228-234
- Klein *et al.* (2003) *Forensic Sci. Comm.* 5(1)

Klein *et al.* (2003) Forensic Sci. Comm. 5(1) **Addressing Ambient Temperature Variation Effects on Sizing Precision of AmpFlSTR® Profiler Plus™ Alleles Detected on the ABI Prism® 310 Genetic Analyzer**

Abstract

Early studies have established the Local Southern algorithm as a precise tool for sizing DNA fragments. As a result, the Local Southern algorithm of the PE Applied Biosystems' software, GeneScan® Analysis (PE Applied Biosystems, Foster City, California), is the manufacturer's recommended method for sizing short tandem repeats (STRs). However, this recommendation is made with the warning that size estimates may be imprecise if any of the standard fragments run anomalously. Specifically, the GeneScan®-500 (GS-500) internal standard fragments of 250 and 340 bases in length run anomalously under non-optimal conditions on the ABI Prism® 310 Genetic Analyzer (PE Applied Biosystems, Foster City, California).

The California Department of Justice DNA Laboratory currently uses the GS-500-size standard without the 250-base standard assigned and the Local Southern method to size AmpFlSTR® Profiler Plus™ alleles. However, even with the manufacturer's recommended instrument running conditions, studies in this laboratory demonstrate that ambient temperature variation over the course of a 310 run can result in anomalous migration of GS-500 standard fragments. When ambient temperature varies, a simple analysis method change can improve precision.

This study suggests that **the Global Southern method may provide improved precision over the Local Southern method when using the GS-500 internal standard with the ABI Prism® 310 Genetic Analyzer**. In addition, this study shows that precision for fragments greater than 300 bases is further improved by excluding the 340-base GS-500 fragment in conjunction with using the Global Southern method. **When ambient temperature shifts occur, this sizing method change should reduce the number of sample reruns necessary**.

http://www.fbi.gov/hq/lab/fsc/backissu/jan2003/klein.htm

Thoughts on Size Standards

- Be consistent in use if you want to be able to compare data over time
- All size standards I have tested work
- Allele sizes are different with different internal sizing standards
- GS500 has a large "hole" in its sizing ability when using the local Southern algorithm for medium-sized STR alleles because of the 250 bp peak that cannot be used; also must be run out to 450 bp to be able to type large FGA alleles with ABI kits

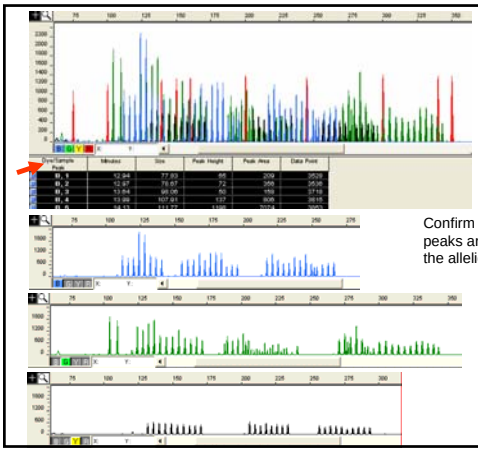
Summary of Steps in GeneScan Data Analysis

Create New Project (Load sample files)
Establish Matrix
Define Size Standard Peaks
Set Analysis Parameters
- Minimum peak height (e.g., 50 RFU)
- Scan range evaluated (best to avoid primer region)
- Sizing algorithm options (Local Southern is default)

Data Review Windows
- Analysis Control Window
- Results Control Window

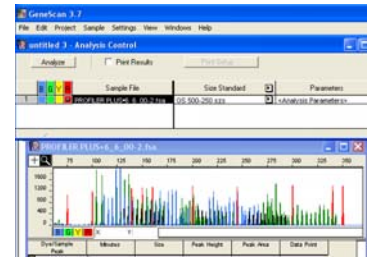
Steps for Processing Samples:
- Install matrix onto each (highlighted) sample
- Select appropriate size standard (or create a new one)
- Check analysis parameter values (or select pre-defined analysis parameter file)
- Click "Analyze" button

Examine data in Analysis Control by double clicking on sample file name (this allows you to pull up files in all colors and select or de-select color of interest)

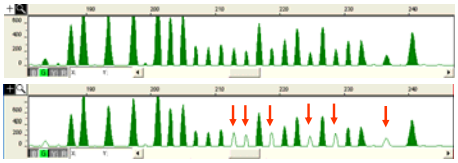


Confirm that all allele peaks are called in the allelic ladder

Effect of Peak Detection Threshold

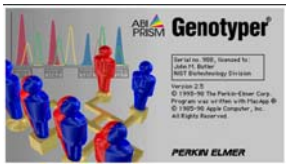


Failure to have these alleles designated will result in Genotyper macro failure and inability to type samples



Peak threshold too high so that some alleles are not designated as peaks by the software

Genotyper Software



Macintosh	Windows NT
2.0	3.7 (5-dye)
2.5	
2.5.2 (5-dye)	

- Converts GeneScan sized peaks into genotype calls using macros
- Genotyping performed by comparison of allele sizes in allelic ladder to sample alleles

ABI manual is P/N 904648

Summary of Genotyper Data Analysis

Open template file containing typing macro specific for STR kit used

Import *PROCESSED/REVIEWED* GeneScan files

Check internal size standard peaks

Run Kazaam (or Power) macro

Review allelic ladder to confirm that all alleles are called correctly

Review sample data by dye color

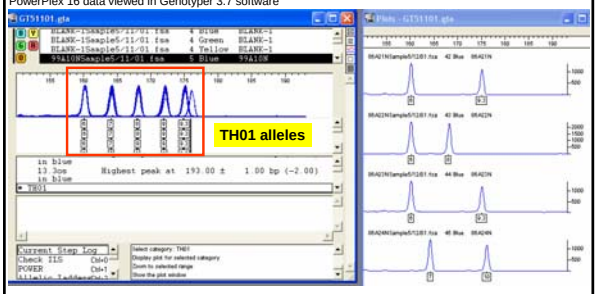
Remove calls to stutter peaks or instrument artifacts

Create allele table

Export allele table

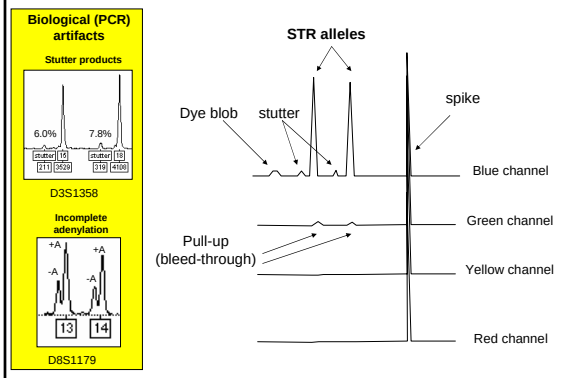
Rapid Genotyper Data Review

PowerPlex 16 data viewed in Genotyper 3.7 software



An overlay of electropherograms permits a rapid assessment of sizing precision and the number of alleles seen in a particular sample set…

Deciphering Artifacts from the True Alleles

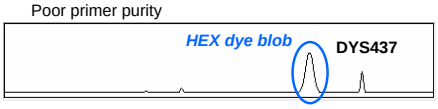


Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 15.4, © Elsevier Science/Academic Press

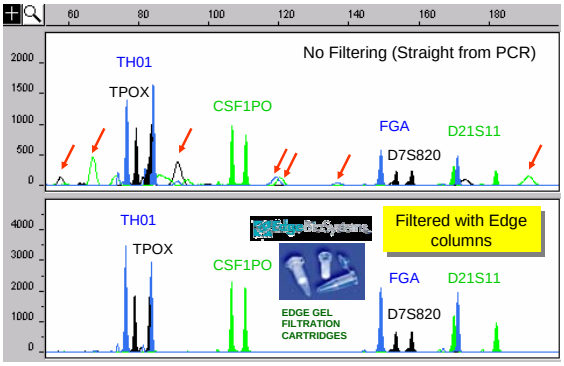
Dye Blobs ("Artifacts")

- Free dye (not coupled to primer) can be injected into the CE capillary and interfere with detection of true STR alleles
- **Dye blobs are wider and usually of less intensity** than true STR alleles (amount depends on the purity of the primers used)
- Dye blobs usually appear at an apparent size that is unique for each dye (e.g., FAM ~120 bp, PET ~100 bp)

Poor primer purity



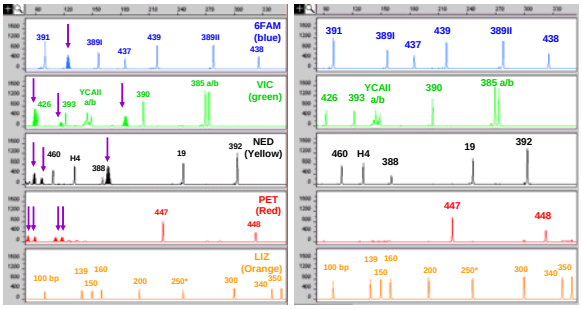
Removal of Dye Artifacts Following PCR Amplification



Butler, J.M., Shen, Y., McCord, B.R. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J. Forensic Sci* 48(5) 1054-1064.

Residual dye artifacts

Dye blob removal with Edge columns



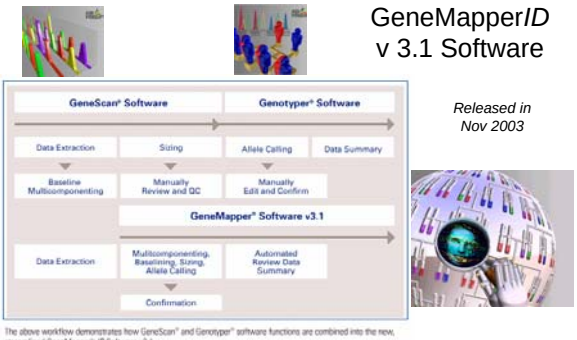
NIST Y-STR 20plex assay

Butler, J.M. (2005) Constructing STR multiplex assays. *Methods in Molecular Biology: Forensic DNA Typing Protocols* (Carracedo, A., ed.), Humana Press: Totowa, New Jersey, *in press*.

Common Errors in Genotyping

- Genotyper table does not import the third allele in a tri-allelic pattern
- Bleedthrough (pull-up) between dye colors results in a peak that falls into a possible allele bin in an adjacent color
- Clicking off a peak label for a true allele by accident and failing to restore the label
- Accidentally clicking on a peak and inserting a label near the beginning of a locus size range that is not a true allele, which causes the second true allele to not show up in the final table of results since only two alleles are imported for each locus

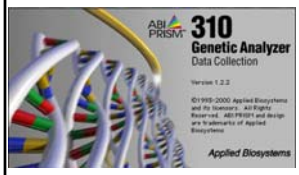
GeneMapper*ID* v 3.1 Software



Released in Nov 2003

Since June 2004, Applied Biosystems no longer sells GeneScan and Genotyper software (they will support these programs until June 2009)…ABI is encouraging the adoption of GeneMapper*ID* which replaces the functions of GeneScan and Genotyper.

310 Data Collection Software and GeneMapper*ID*



Macintosh	Windows NT
1.0.2	1.0 (5-dye)
1.2.2	2.0 (5-dye)
2.1 (5-dye)	

Only compatible with GeneMapper software (not back compatible with GeneScan/Genotyper)

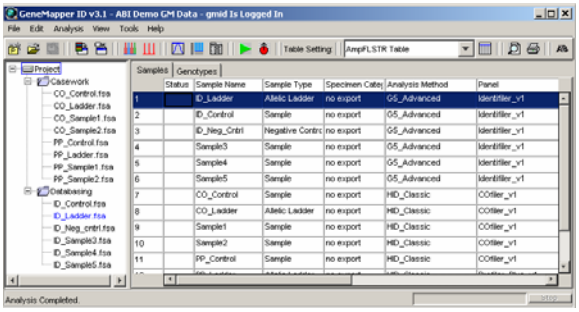
- Controls 310 run conditions
- Translates light on CCD camera into electropherogram (raw data)
- Sample sheets and injection lists are created

ABI manual is P/N 904958B

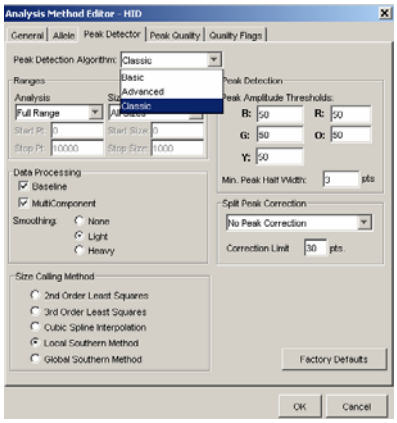
Use of GeneMapper*ID* v3.1

- Projects
- Kit, panel, marker, bin
- Provides a flexible format and different methods for viewing data
- Can analyze results from multiple STR kits within the same GeneMapper project file
- Not designed to work with pentanucleotide repeat loci (version 3.2 will be able)

The Project Window



Analysis Parameters Window



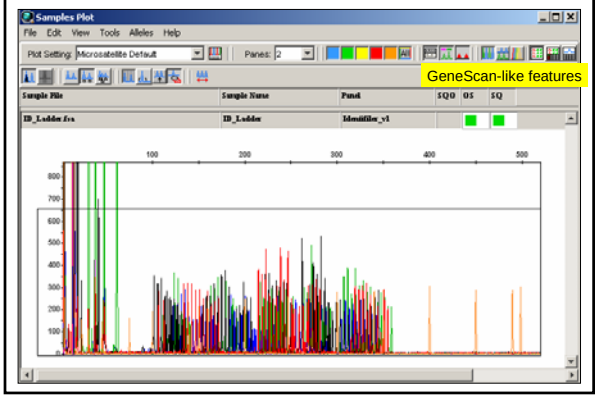
Algorithms:

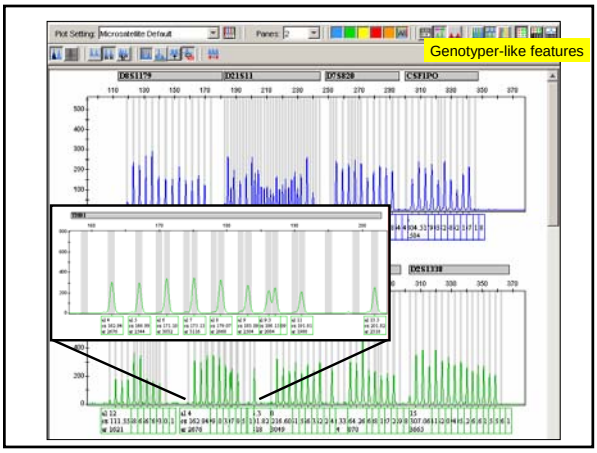
Basic is the default for many applications

Advanced is similar to the Windows NT version of GeneScan

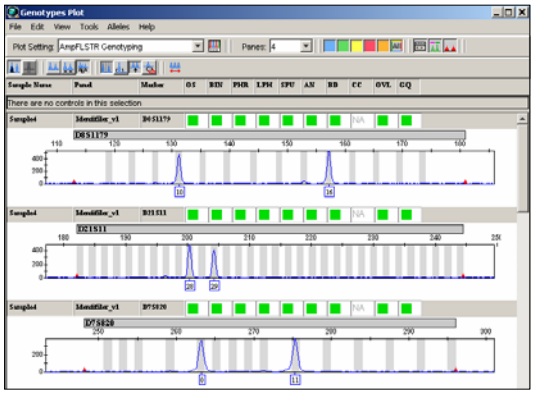
Classic is similar to Macintosh version of GeneScan

Sample Plot Window

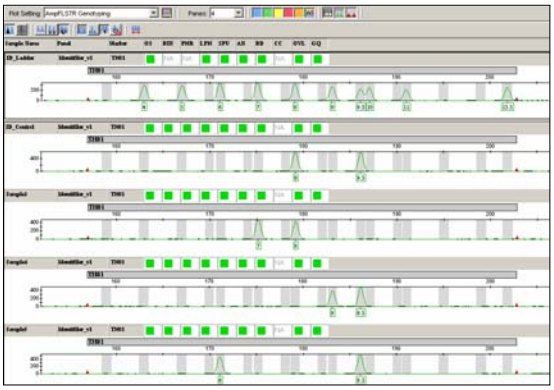




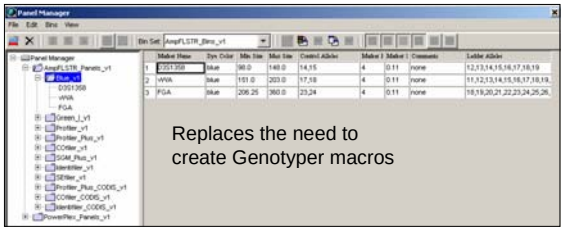
Review Multiple Loci from a Single Sample



Review Multiple Samples at a Single Locus



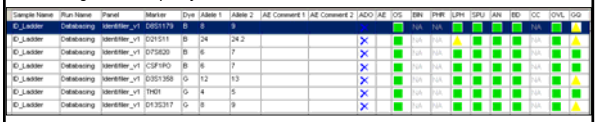
Panel Manager Window



A kit is a collection of panels.
A panel is a collection of markers.
Bin sets are collections of the expected allele locations for markers contained with a kit.

Process Component Based Quality Values (PQVs)

Green squares = pass
Yellow triangles = check
Red octagons = low quality data



ADO = Allele Display Overflow
OS = Offscale
AE = Allele Edit
LPH = Low Peak Height
SPU = Spectral Pull-Up
AN = Allele Number
BD = Broad Peak
OVL = Overlap
GQ = Genotype Quality

Expert Systems Under Development

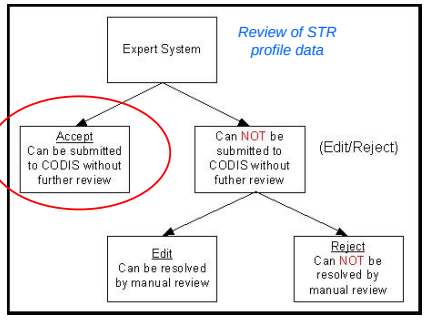
- Difference between an “expert system” and a “quality assurance” tool

Examples

- **TrueAllele** (Cybergenetics, Pittsburg, PA)
  - Kadash, K. *et al.* (2004) *J. Forensic Sci.* 49(4):660-667
- STRess2 (Forensic Science Service, UK)
- OSIRIS (NCBI/NIJ, Steve Sherry’s team)

- **CompareCalls** (Myriad Genetics, Salt Lake City, UT)
  - Ryan, J.H. *et al.* (2004) *J. Forensic Sci.* 49(3):492-499

Model for Future Use of Expert Systems



NDIS Board Appendix B

TrueAllele

cybergenetics

Journal of Forensic Sciences (July 2004), volume 49(2), pp. 660-667

J Forensic Sci, July 2004, Vol. 49, No. 4
Paper ID JFS2003336
Available online at: www.astm.org

Kristy Kadash,[1] Ph.D., D-ABC; Brian E. Kozlowski,[1] M.F.S; Lisa A. Biegg,[2] M.S.; and Barry W. Duceman,[3] Ph.D.

Validation Study of the TrueAllele® Automated Data Review System*

- TrueAllele® is designed to operate independently of other allele calling systems
- A single reviewer with TrueAllele may be used in place of the current two-person review process
- Examined a dataset of 2,048 convicted offender STR profiles and found only four samples with differences between TrueAllele and Genotyper at a single locus each (spikes, calling alleles outside of ladder range, and Genotyper missing the third allele in a tri-allelic pattern)

Presenting Information Collected on the ABI 310

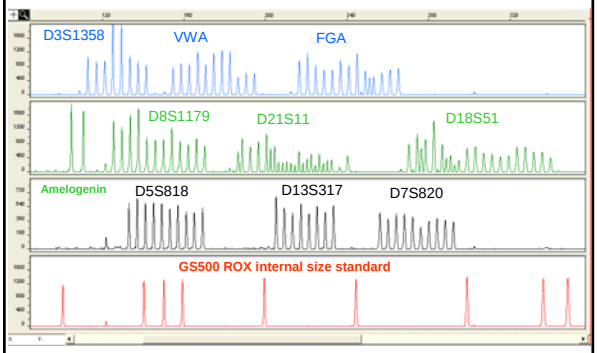
Steps in Creating PowerPoint Files from GeneScan and Genotyper Data

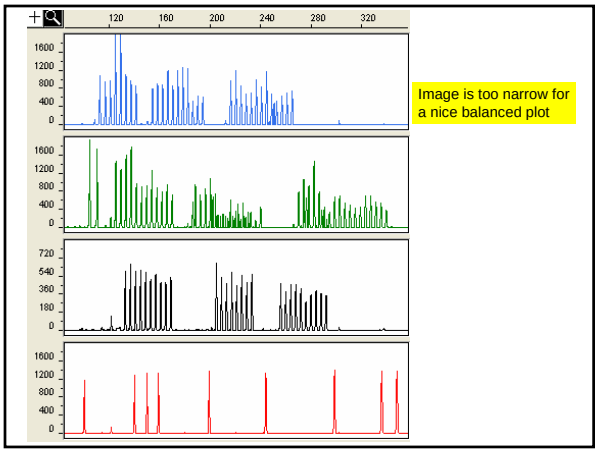
Steps for Putting STR Data into PowerPoint Presentations

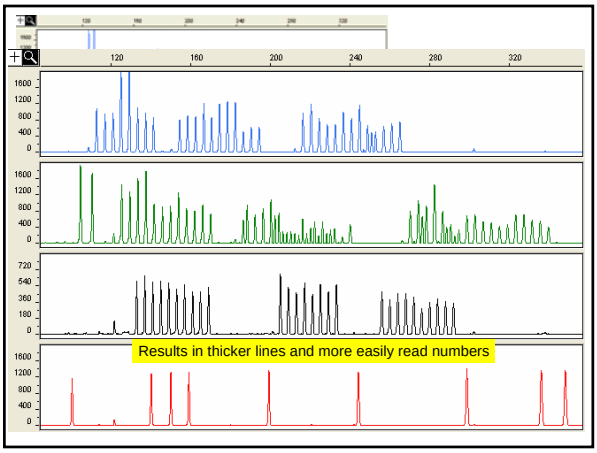
- Open GeneScan or Genotyper file and put data into desired display format
  - It is better to keep image a medium size that can be stretched within PowerPoint to make the lines thicker
- Take a picture of the desired image on screen
  - Windows: press "print screen", paste image into Paint program, then crop portion of image desired
  - Mac: shift-Apple-4 keys, draw box around desired image, open picture image under Macintosh hard drive
- Open PowerPoint and paste copied image from Paint
- Label image within PowerPoint
- Print as 2-per-page handout to have a nice size annotated figure

Example with Profiler Plus Allelic Ladder

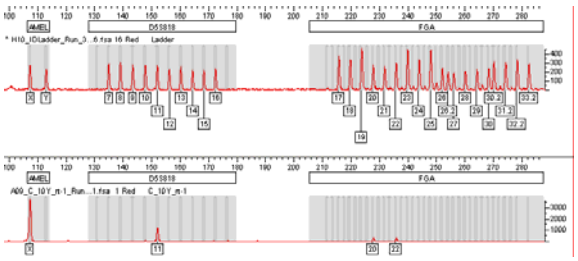
Label Loci…







Example Identifiler Data



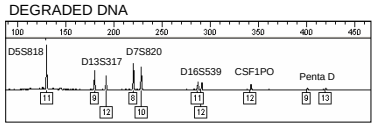
The same thing is done in Genotyper—display image in software, print screen, crop within Paint and paste into PowerPoint, stretch to fill slide, add labels as needed…

Databank vs Casework Data Challenges

- Databank (single source samples)
  - Too much DNA may be added to the PCR reaction resulting in pull-up between dye colors
  - Lots of data to review – often produced by contractors
- Casework (mixtures or low level samples)
  - Often limited DNA material to work with
  - Low copy number samples can result in allele dropout
  - Can produce complicated STR profiles to interpret

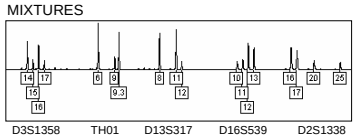
Common Casework Challenges

DEGRADED DNA



Loss of signal at larger size loci

MIXTURES

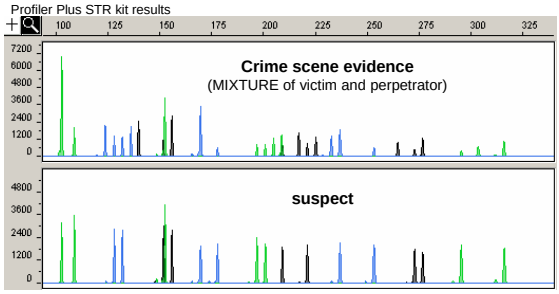


More than two alleles at multiple loci

From Butler, J.M. (2004) Short tandem repeat analysis for human identity testing. *Current Protocols in Human Genetics*, John Wiley & Sons, Hoboken, NJ, Unit 14.8, *(Supplement 41)*, pp. 14.8.1-14.8.22

Forensic Casework DNA Profiles

Profiler Plus STR kit results



All of the suspect's alleles are included in the evidentiary DNA profile