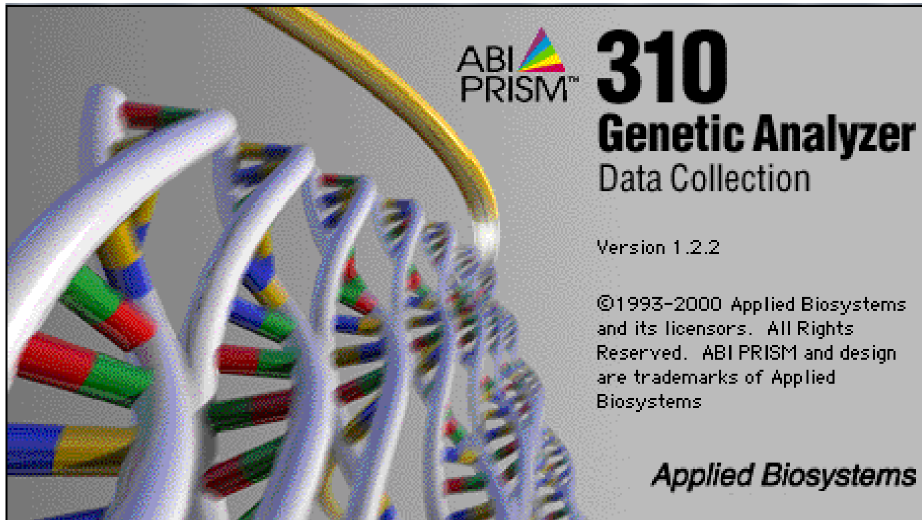
310 Data Collection Software



Macintosh	Windows NT
1.0.2	**Just being released**
1.2.2	
2.1 (5-dye)	

- 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

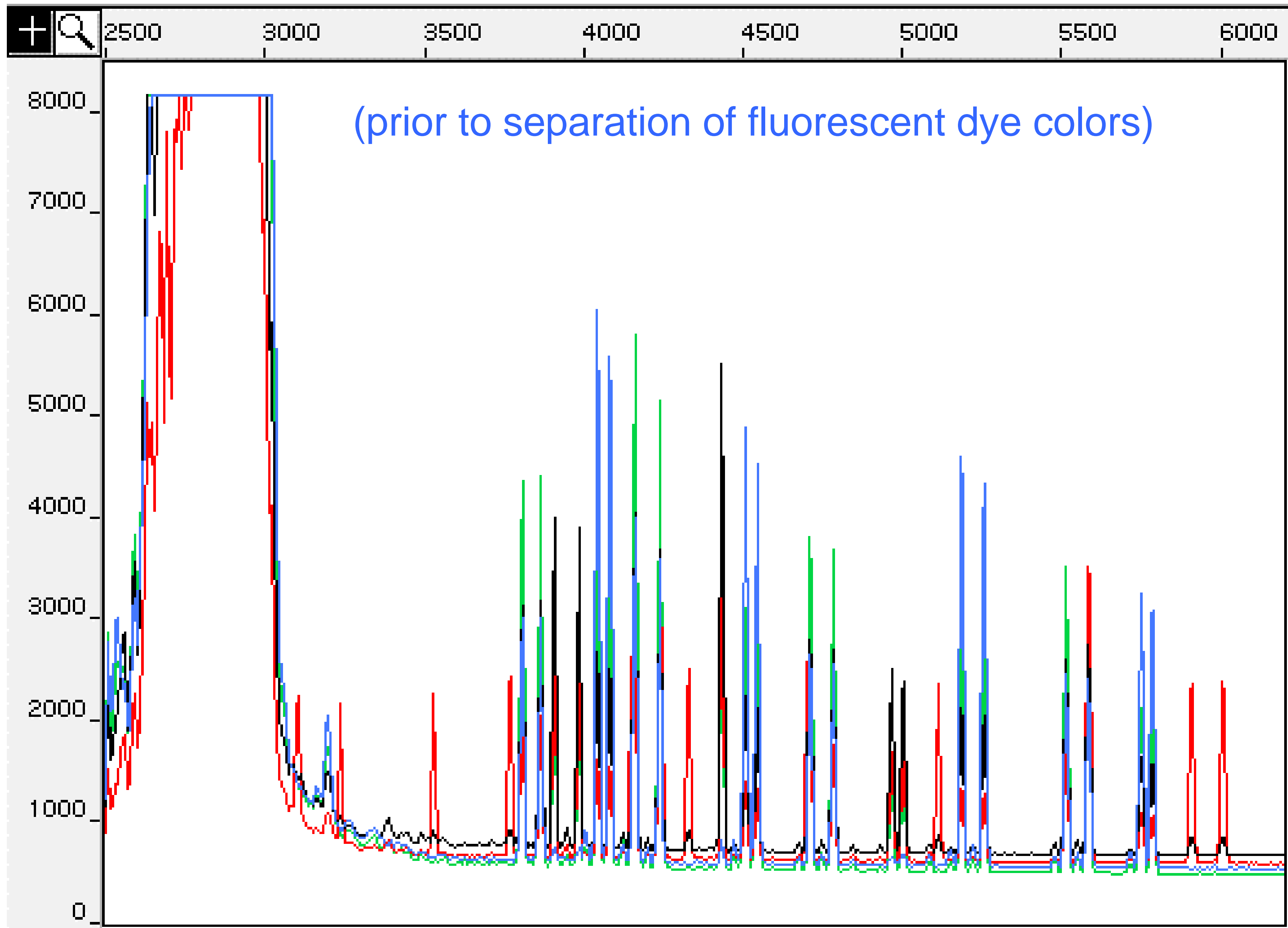
Injection List in Data Collection Software

- Lists samples to be analyzed (repeats can be easily performed)
- Sets virtual filter on CCD camera
- Sets electrophoresis time and voltage
- Sets injection time and voltage
- Sets run temperature
- If desired, sample analysis can be set up for automatic matrix color separation and sizing with internal standards using defined analysis parameters

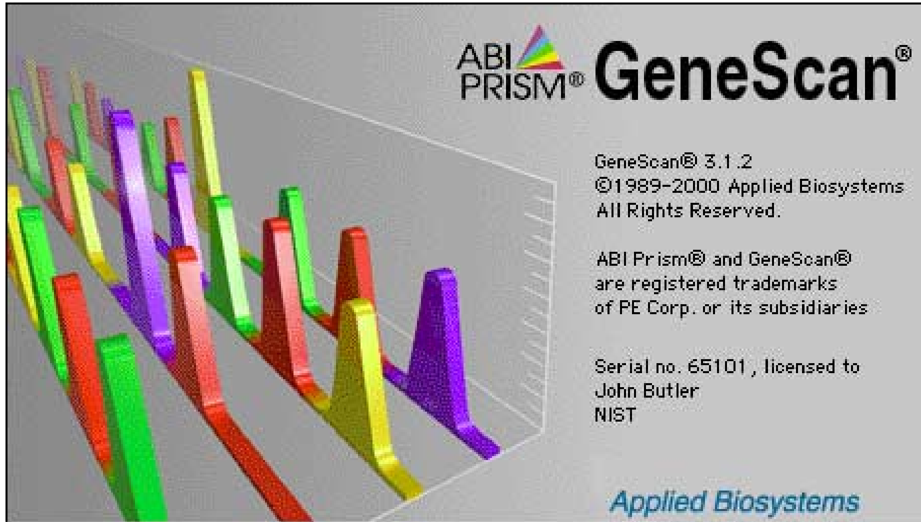
Steps Performed in Standard Module

- **Capillary fill** – polymer solution is forced into the capillary by applying a force to the syringe
- **Pre-electrophoresis –** the separation voltage is raised to 10,000 volts and run for 5 minutes;
- **Water wash of capillary –** capillary is dipped several times in deionized water to remove buffer salts that would interfere with the injection process
- **Sample injection** – the autosampler moves to position A1 (or the next sample in the sample set) and is moved up onto the capillary to perform the injection; a voltage is applied to the sample and a few nanoliters of sample are pulled onto the end of the capillary; the default injection is 15 kV (kilovolts) for 5 seconds
- **Water wash of capillary** – capillary is dipped several times in waste water to remove any contaminating solution adhering to the outside of the capillary
- **Water dip** – capillary is dipped in clean water (position 2) several times
- **Electrophoresis** – autosampler moves to inlet buffer vial (position 1) and separation voltage is applied across the capillary; the injected DNA molecules begin separating through the POP-4 polymer solution
- **Detection** – data collection begins; raw data is collected with no spectral deconvolution of the different dye colors; the matrix is applied during Genescan analysis

Raw Data from the ABI Prism 310



GeneScan® Software



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

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

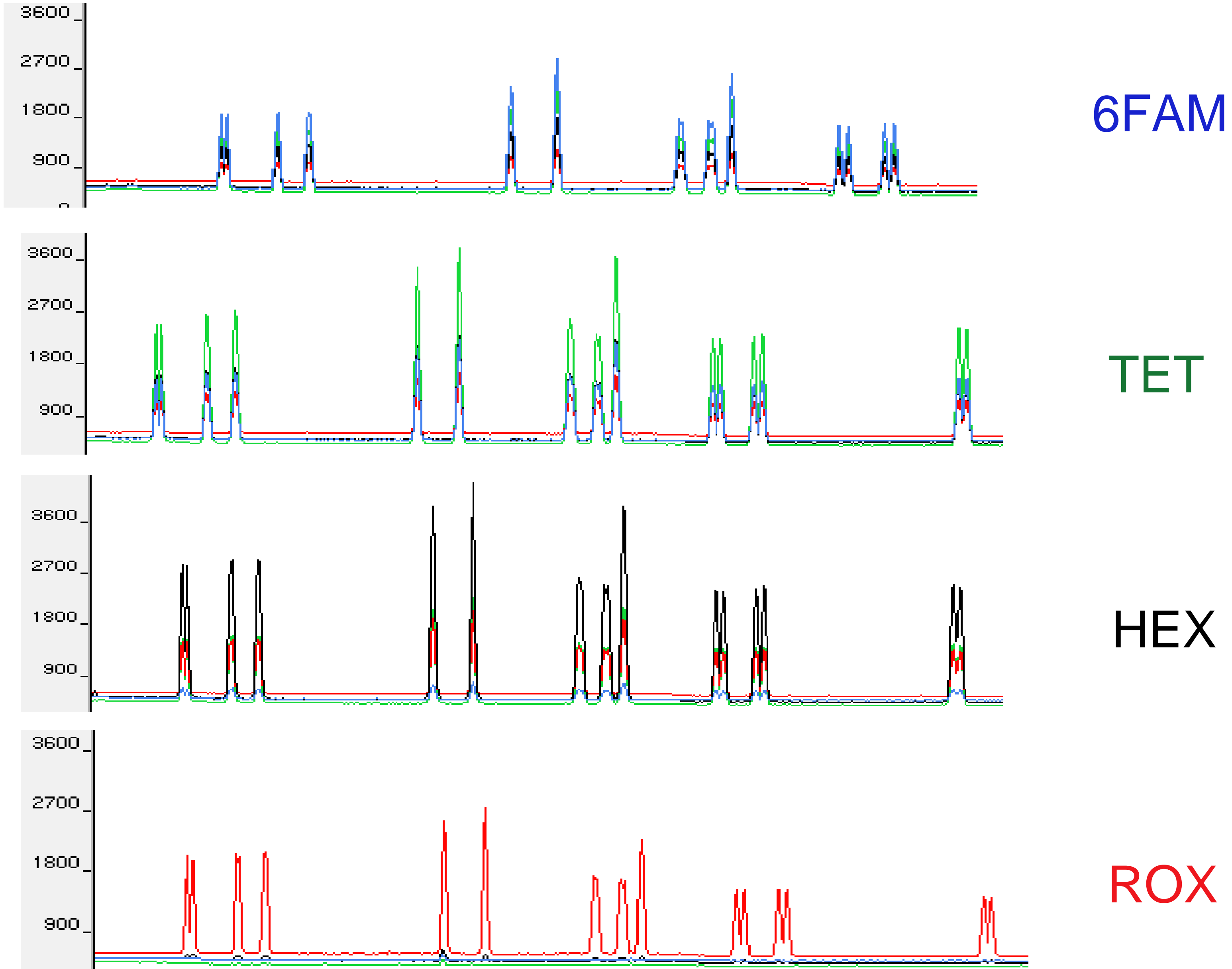
ABI manual is P/N 4303189

Screens in GeneScan® Program

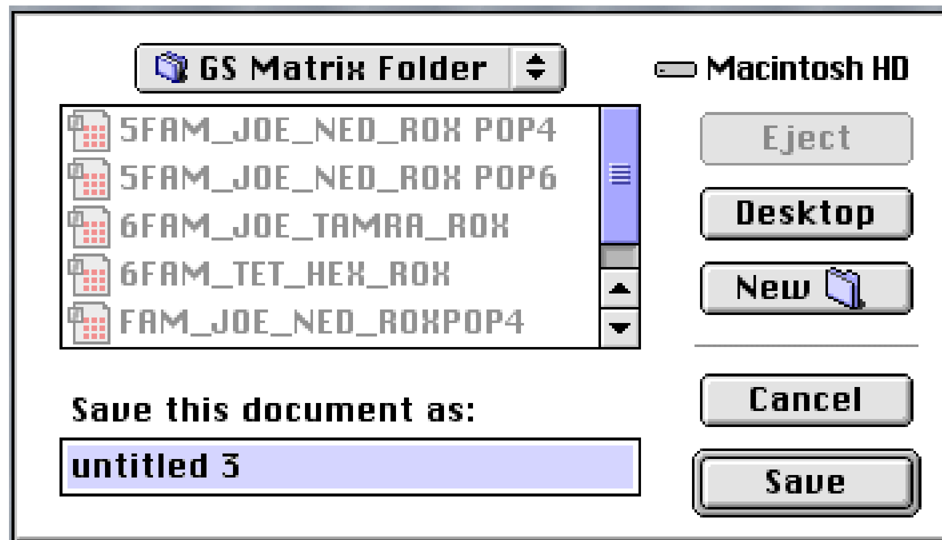
- Processed data
- Sizing data
- Electrophoresis history
- Sample Information
- Raw data
- Analysis log file

Each screen can be used to aid in evaluation of samples and trouble shooting problem samples during data analysis

Matrix Standards (Raw Data)

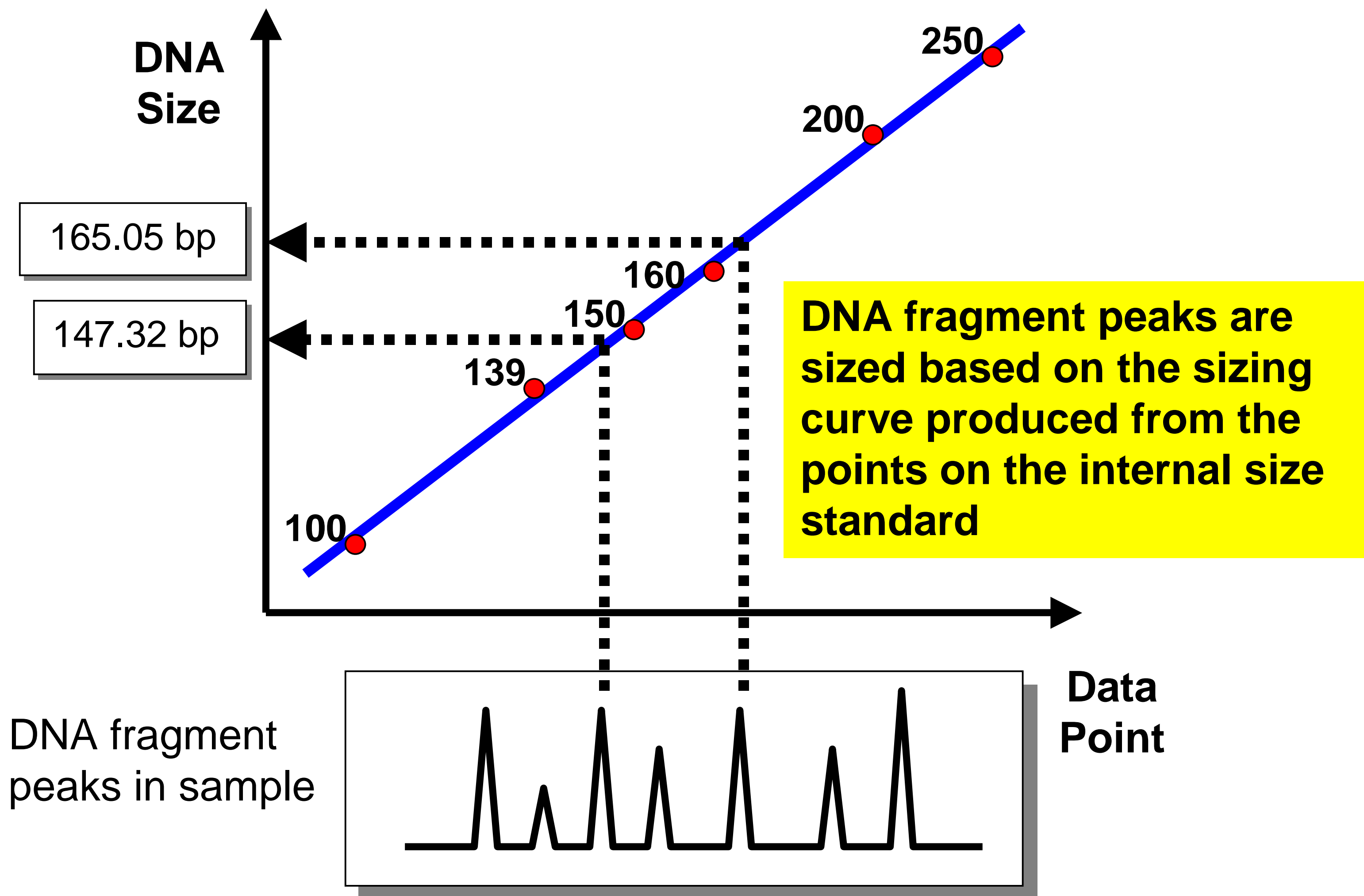


Save 4 x 4 Matrix Created



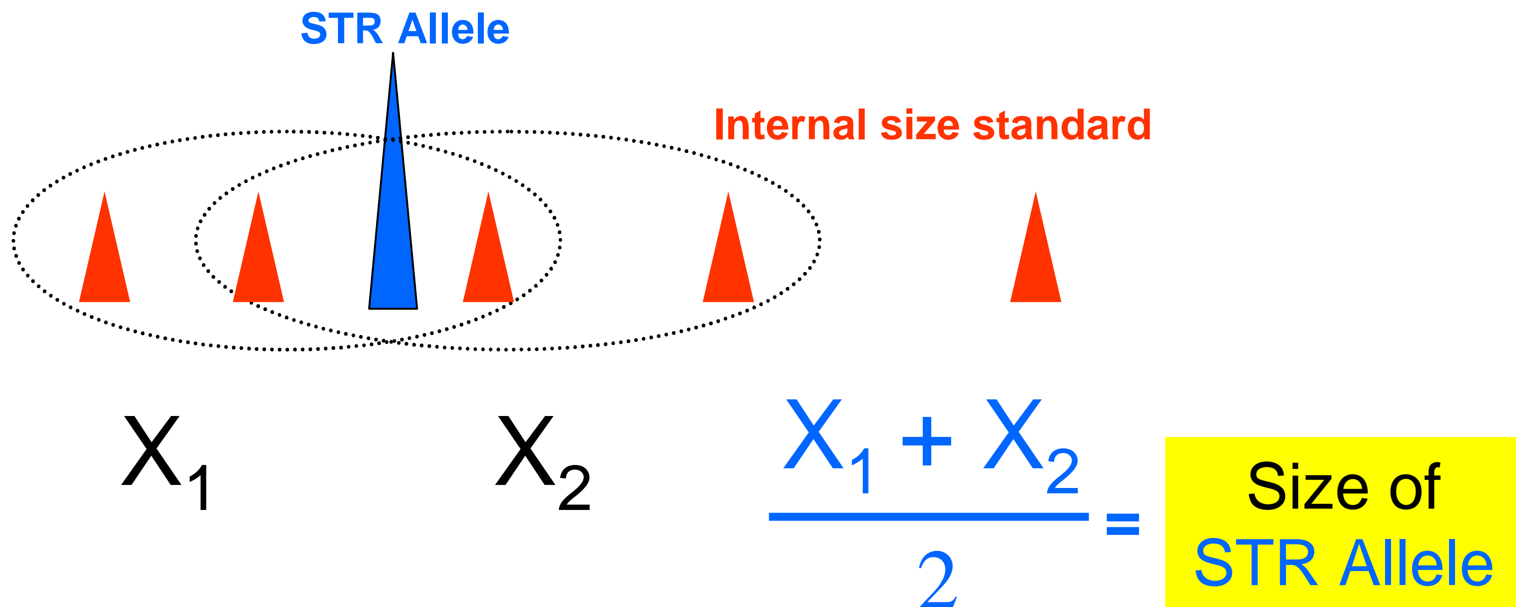
	B	G	Y	R
B	1.0000	0.5194	0.0815	0.0032
G	0.8082	1.0000	0.4959	0.0049
Y	0.5487	0.5353	1.0000	0.0437
R	0.2515	0.3116	0.3887	1.0000

Process of Sizing DNA Fragments Using an Internal Standard



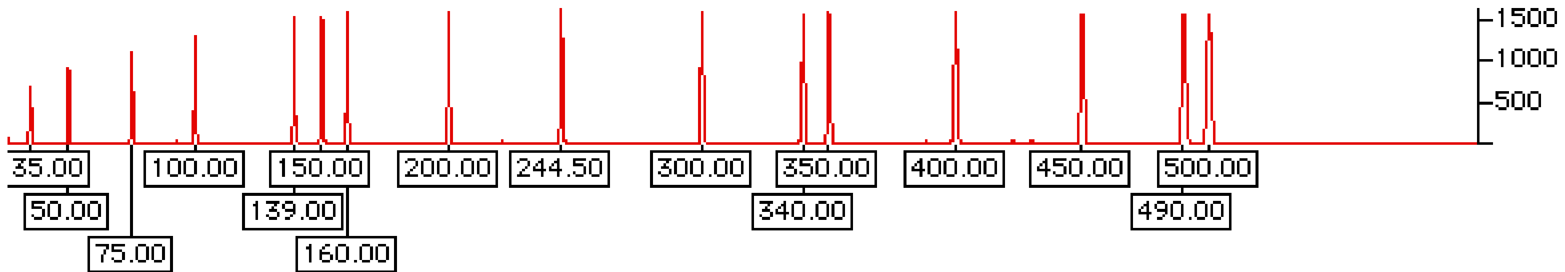
Sizing Algorithm

- 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

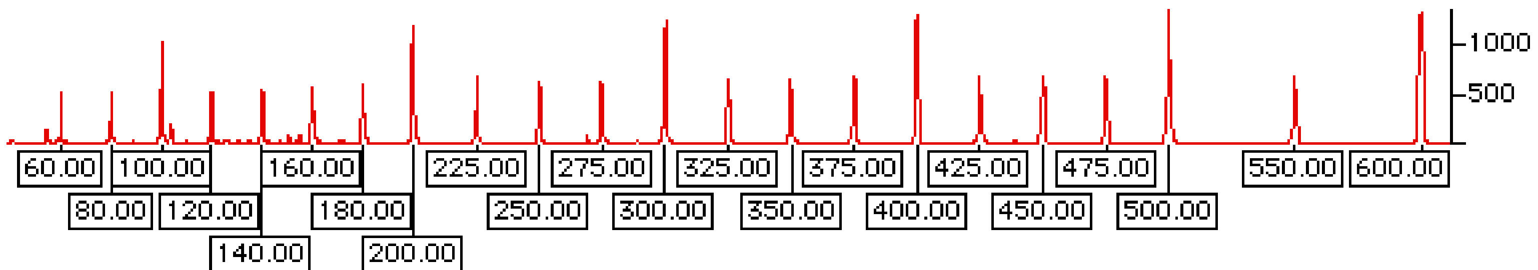


Internal Sizing Standards

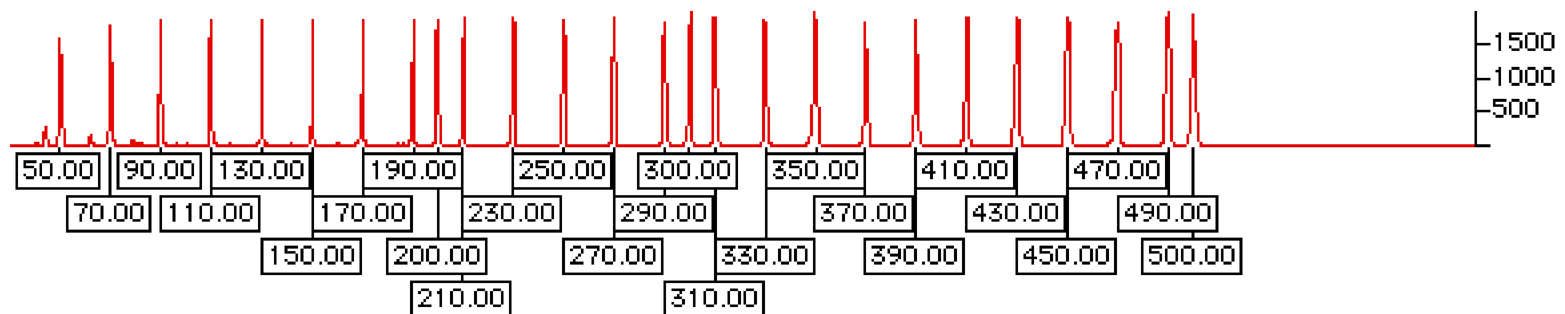
GS500 ROX (Applied Biosystems)



ILS600 CXR (Promega)



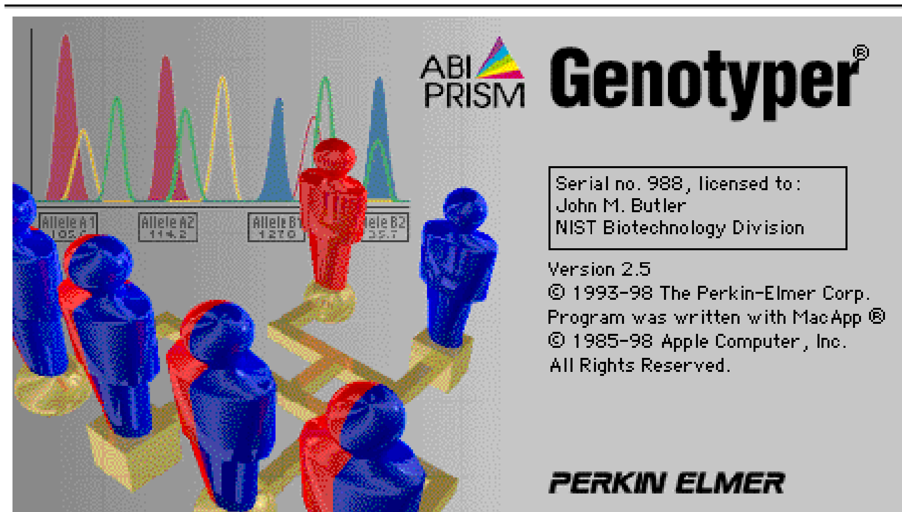
LTI 50-500 ROX (Life Technologies)



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

Genotyper Software

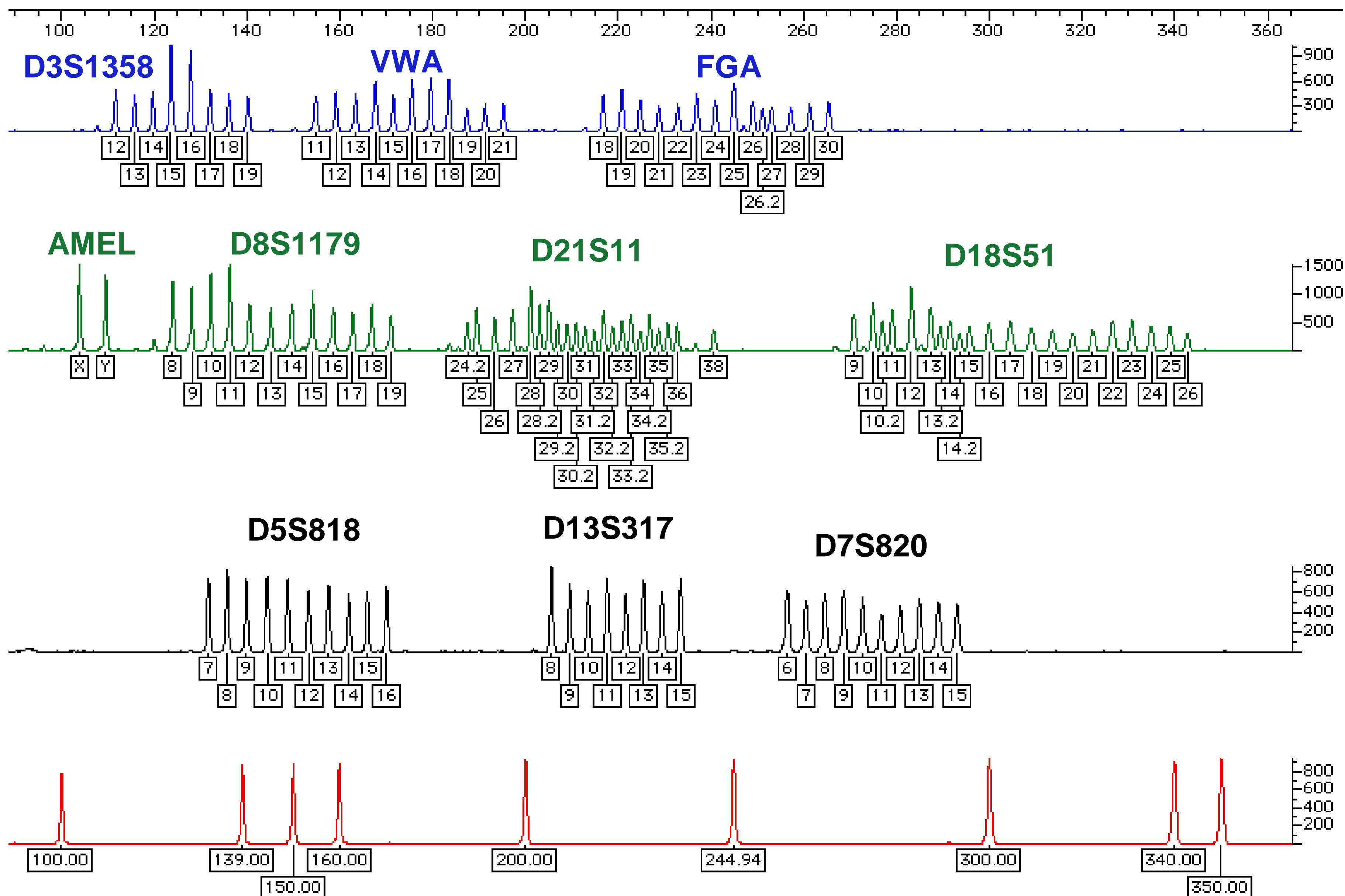


Macintosh	Windows NT
2.0	3.7 (5-dye)
2.5	
2.5.2 (5-dye)	

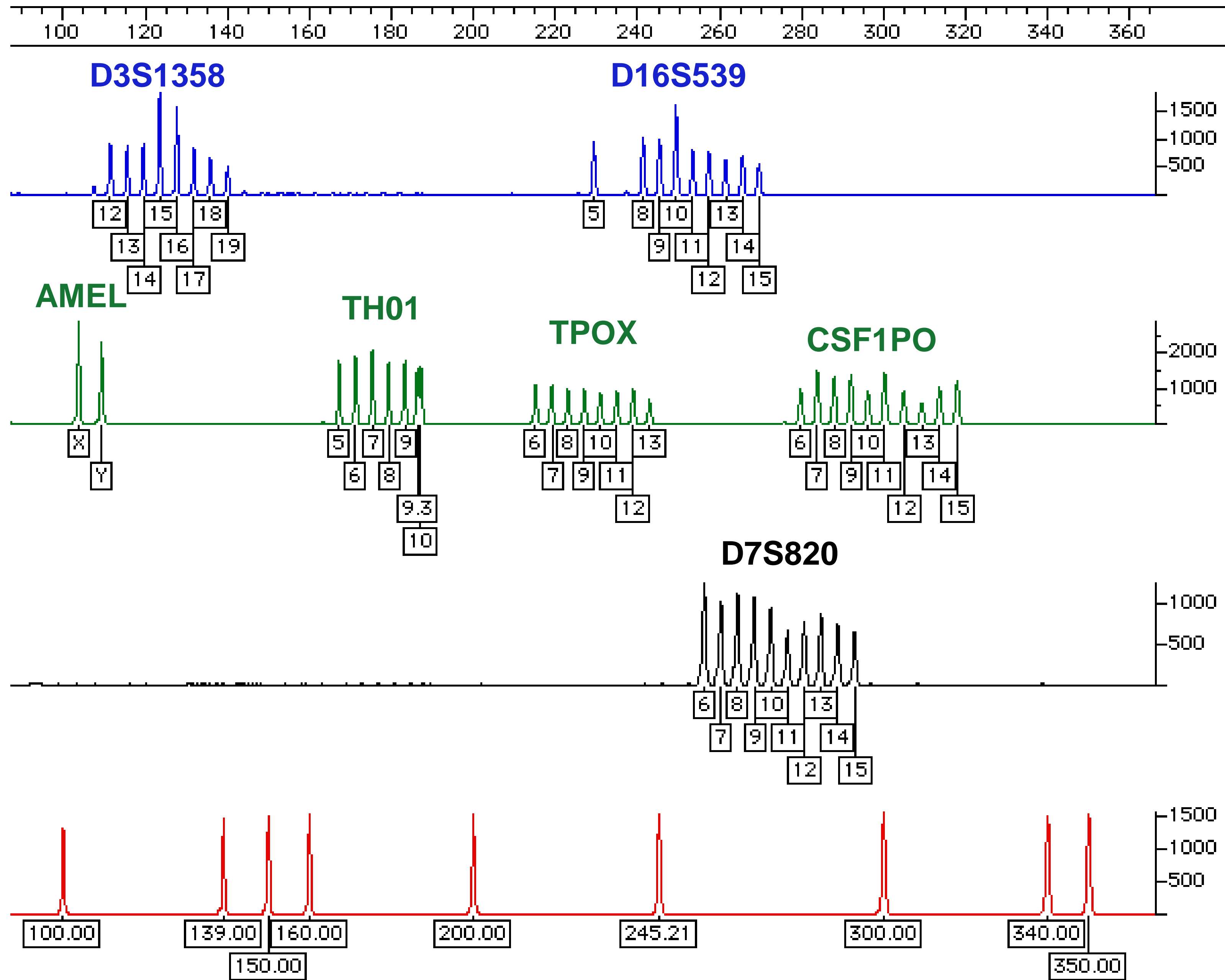
- Converts GeneScan sized peaks into genotype calls using macros
- Genotyping performed by comparison of allele sizes in allelic ladder to sample alleles

ABI manual is P/N 904648

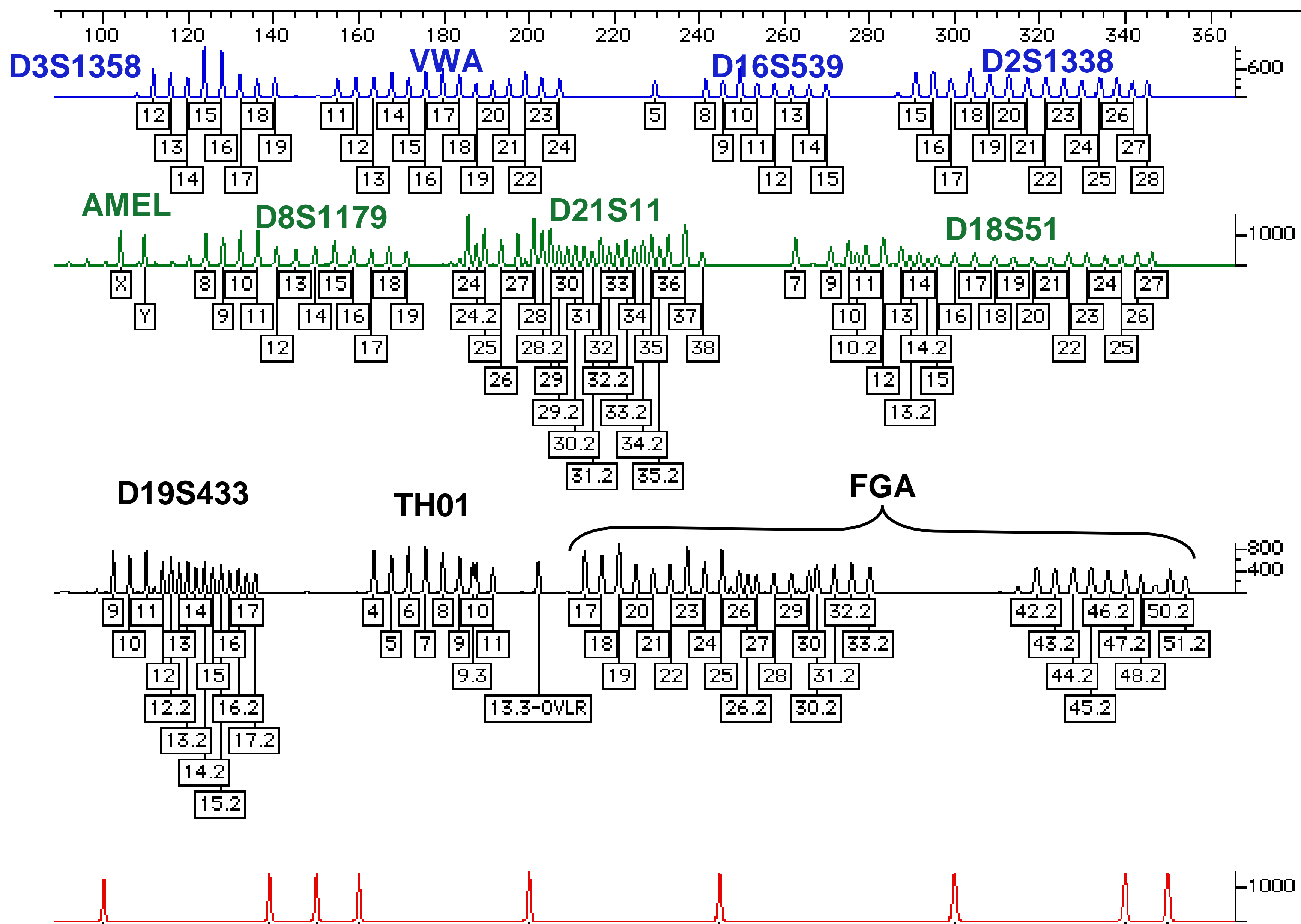
Profiler Plus Allelic Ladders



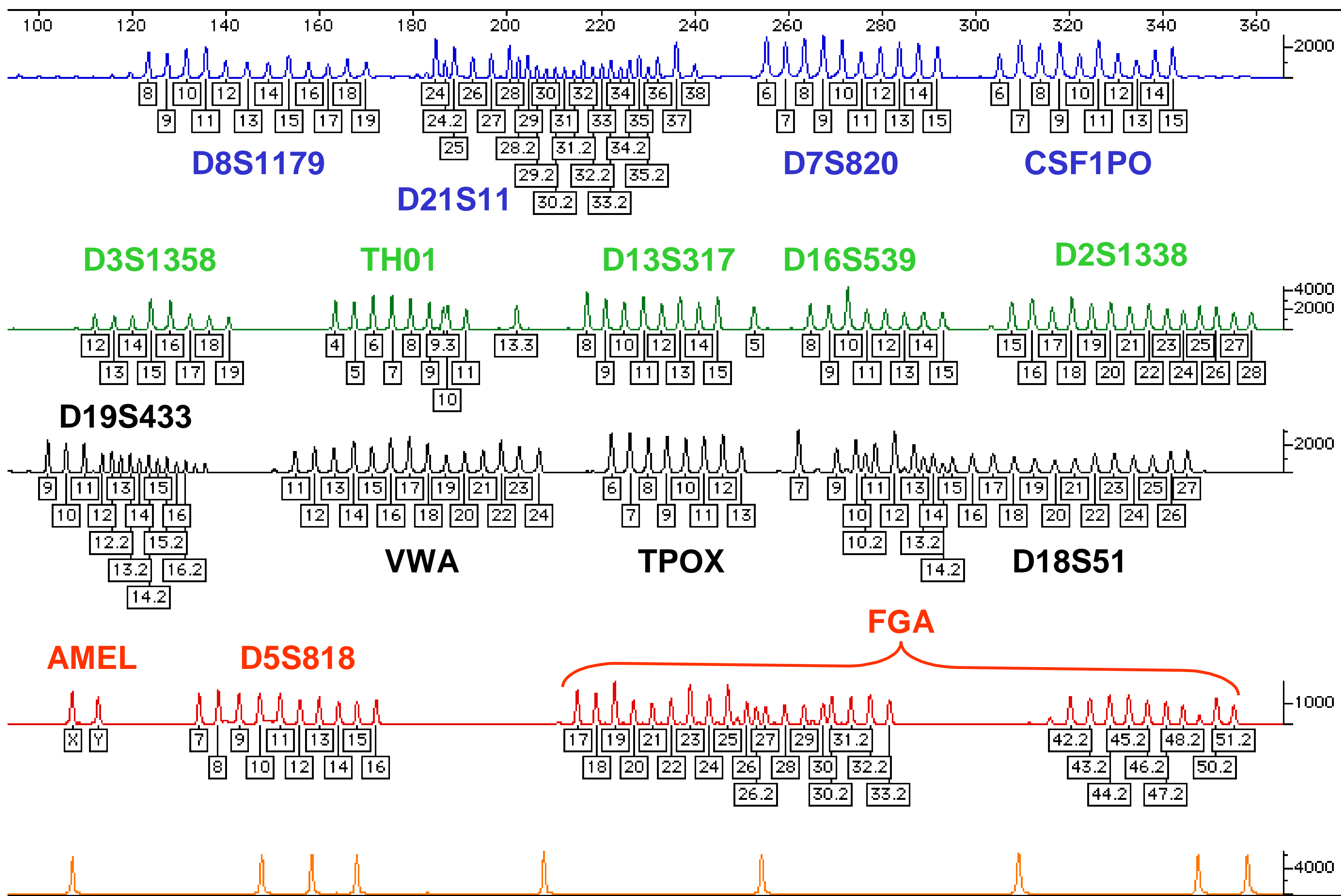
COfiler Allelic Ladders



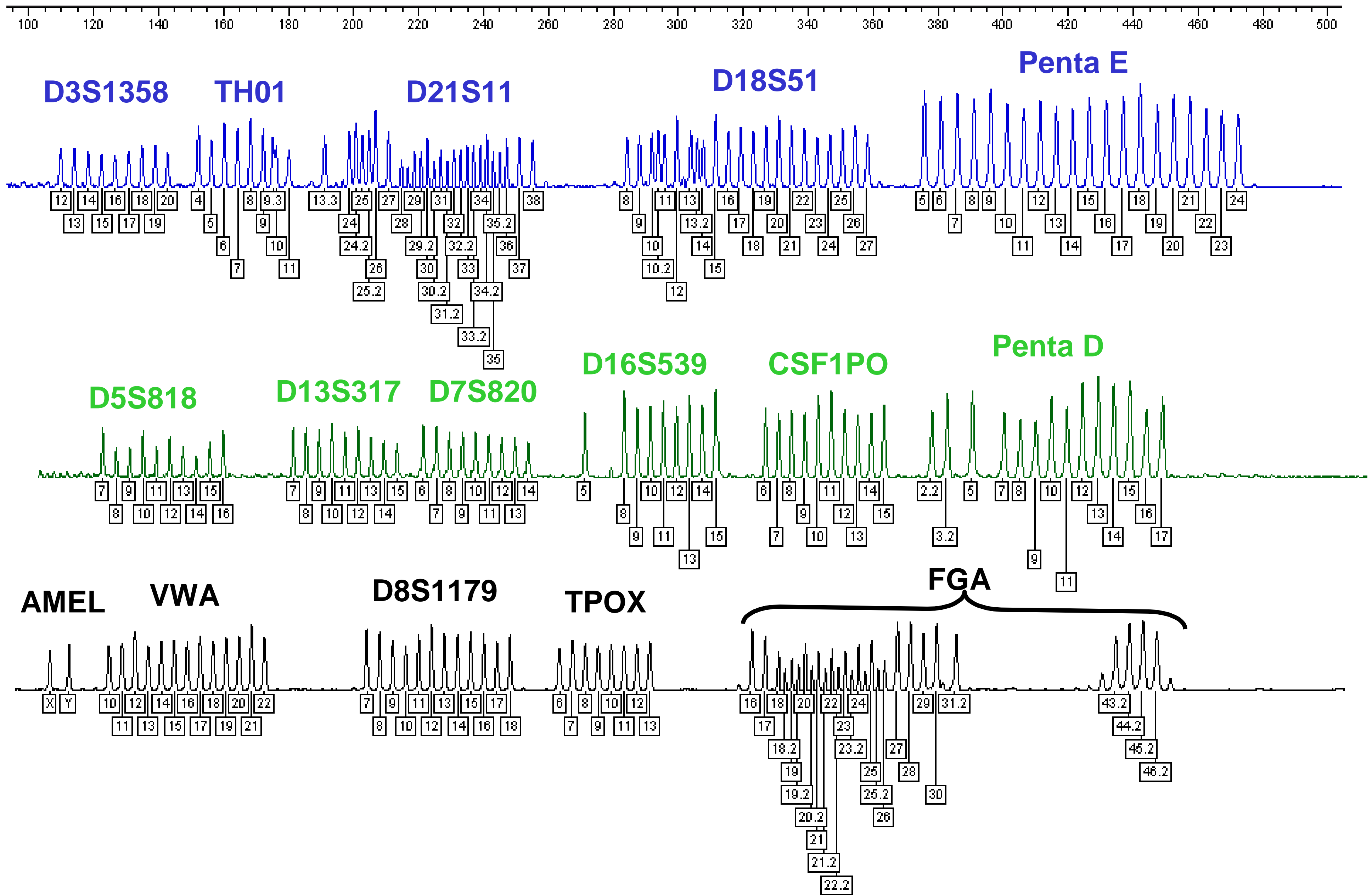
SGM Plus Allelic Ladders



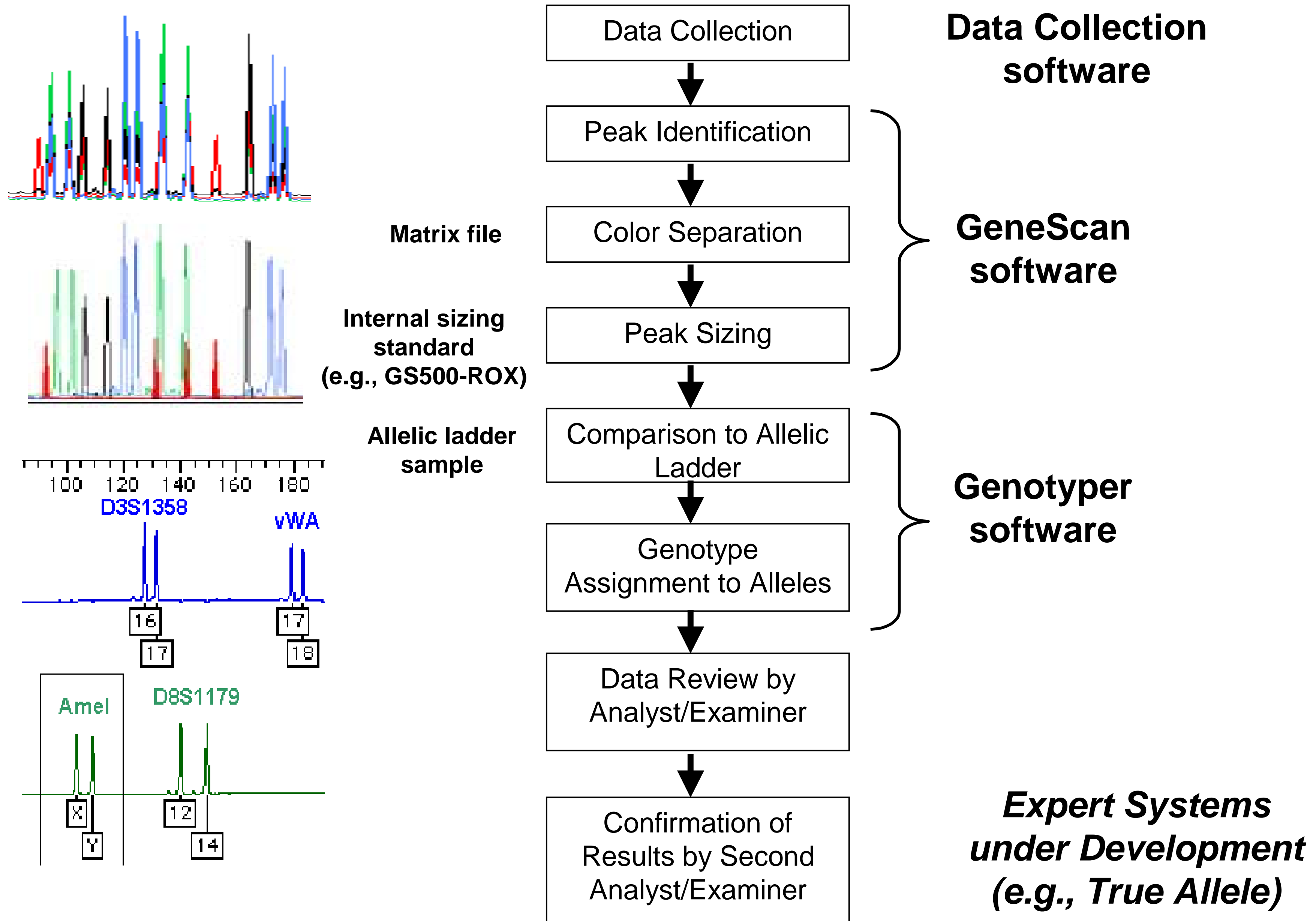
Identifiler Allelic Ladders



PowerPlex® 16 Ladders



Steps in STR Genotyping Process



Three Possible Outcomes

- **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

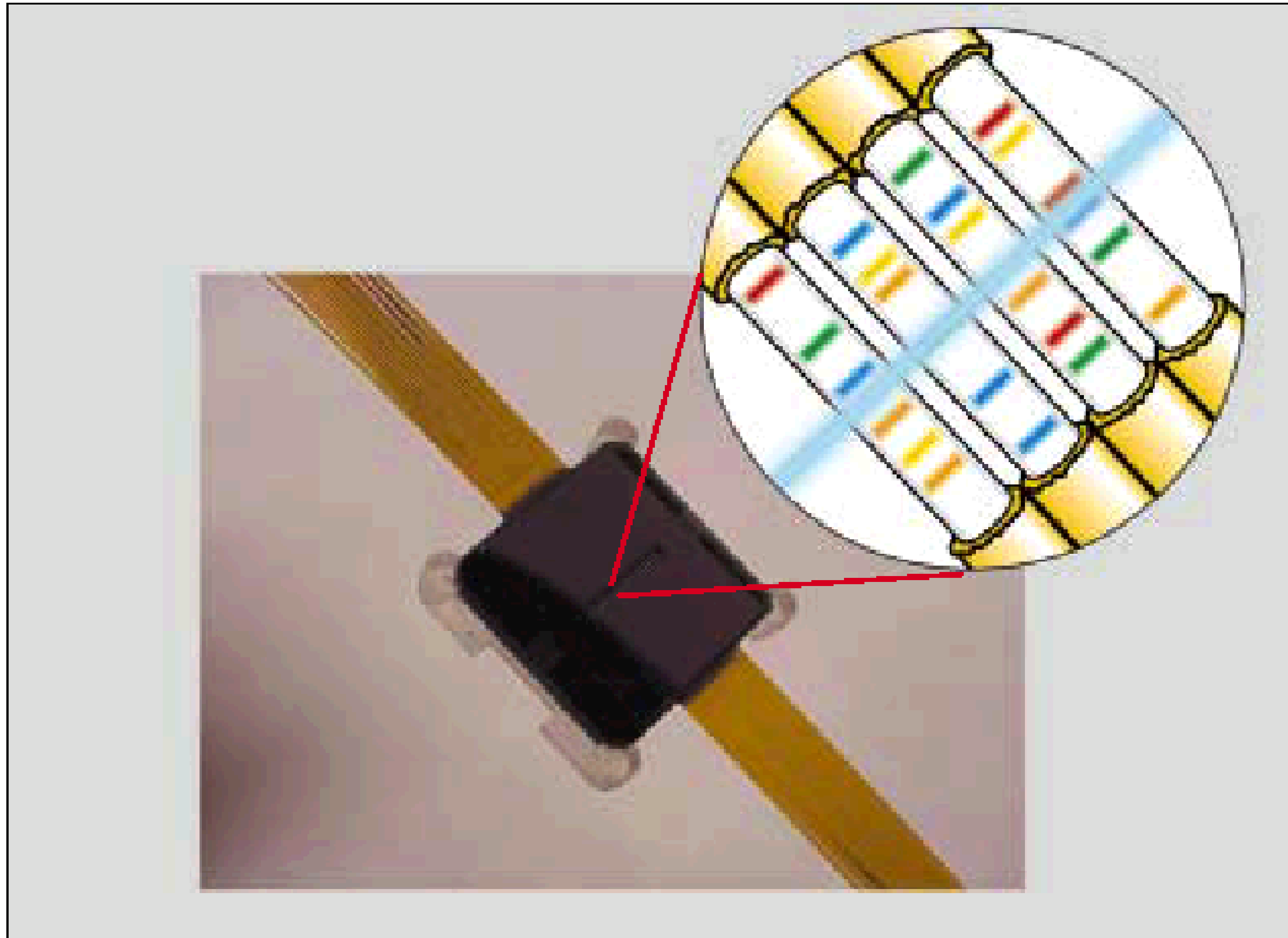
Increasing Sample Throughput with Parallel Processing

ABI 3100
16-capillary array

Subtle differences in matrix formation and sizing algorithms – NOT directly equivalent to 310

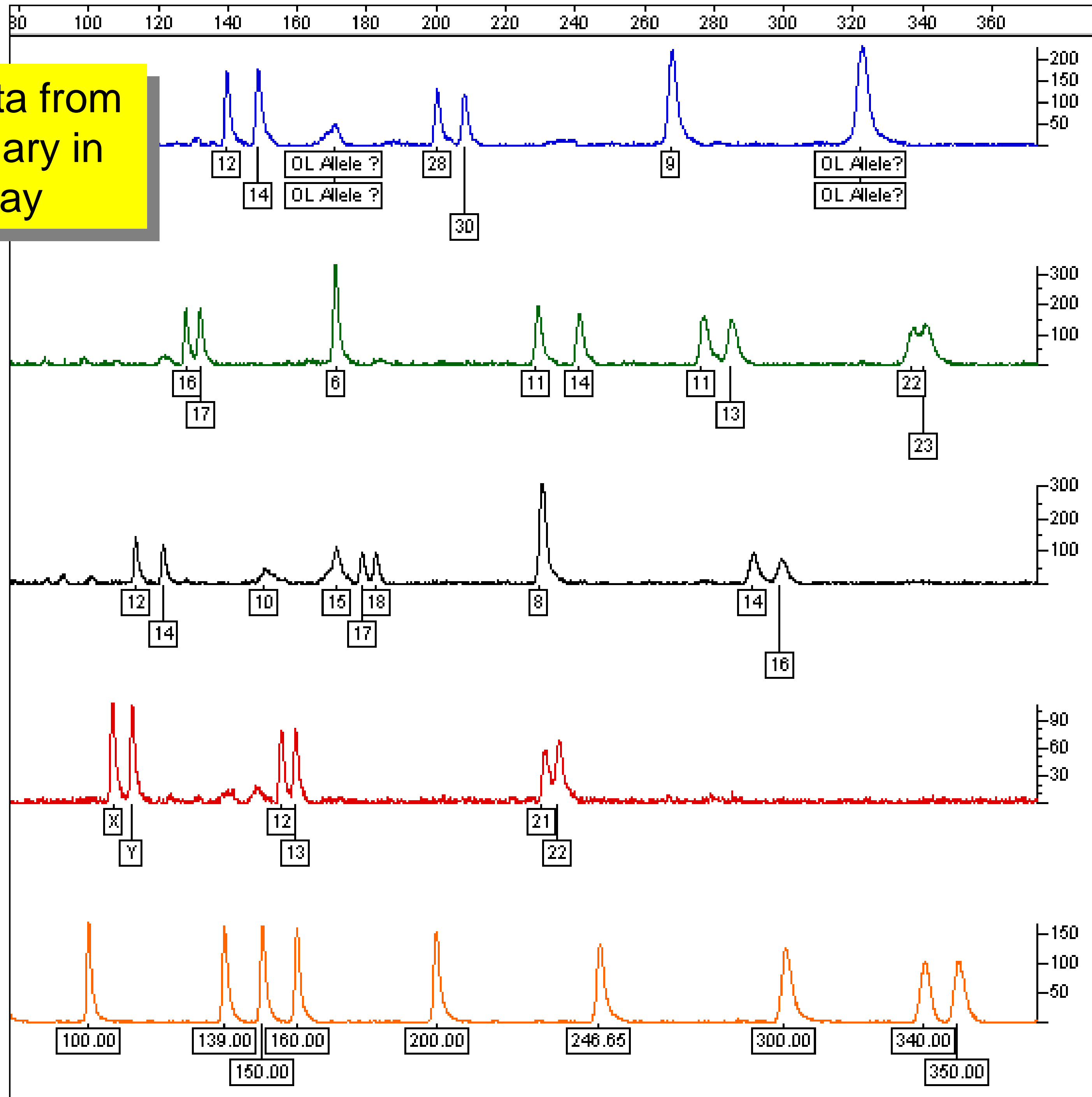
ABI 310
single capillary

ABI 3100 Array Detection

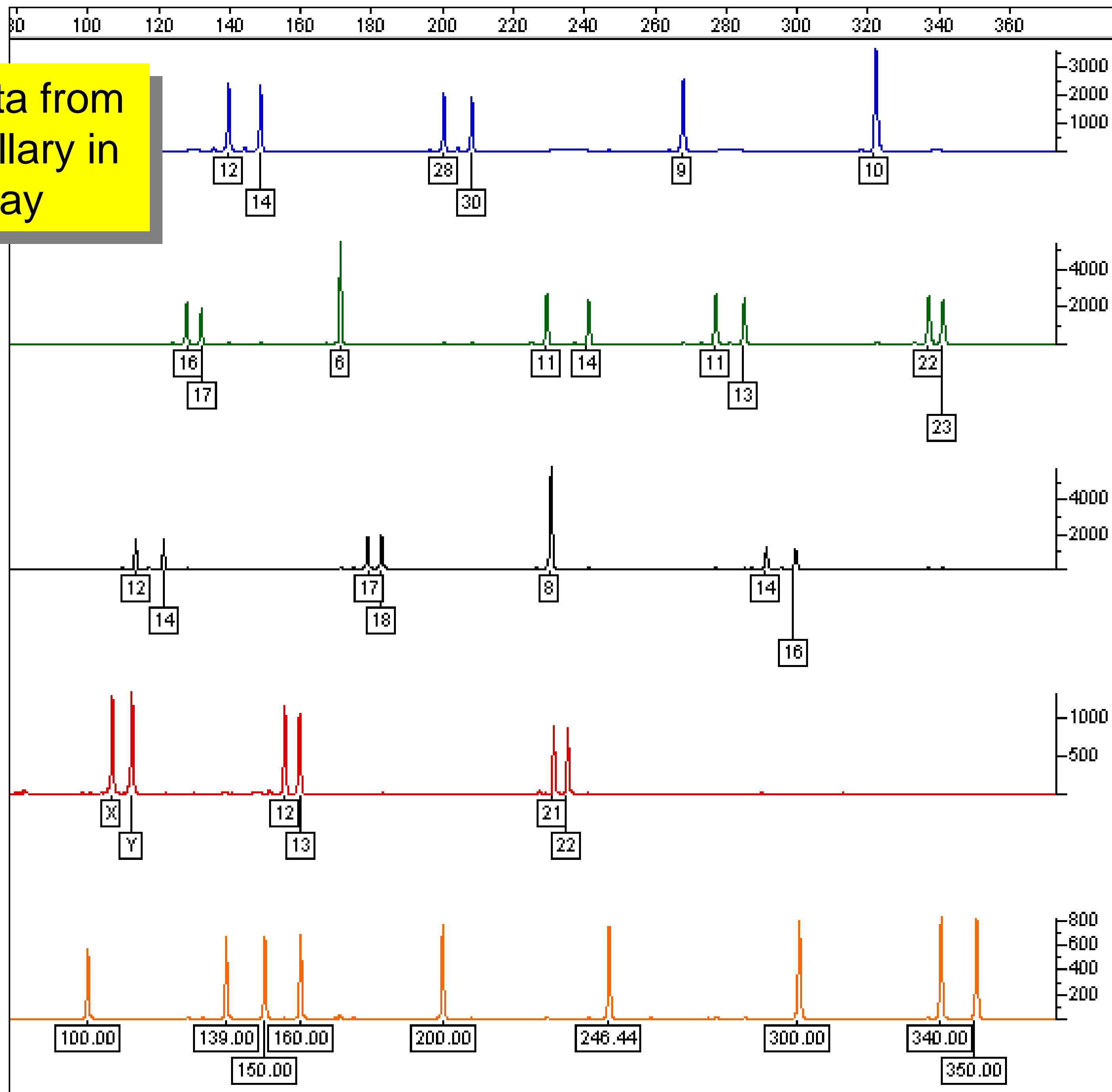


Close-up image of capillary array detection cell with a stylized schematic representation of in-capillary detection.

Identifiler Data from a Bad Capillary in 3100 Array



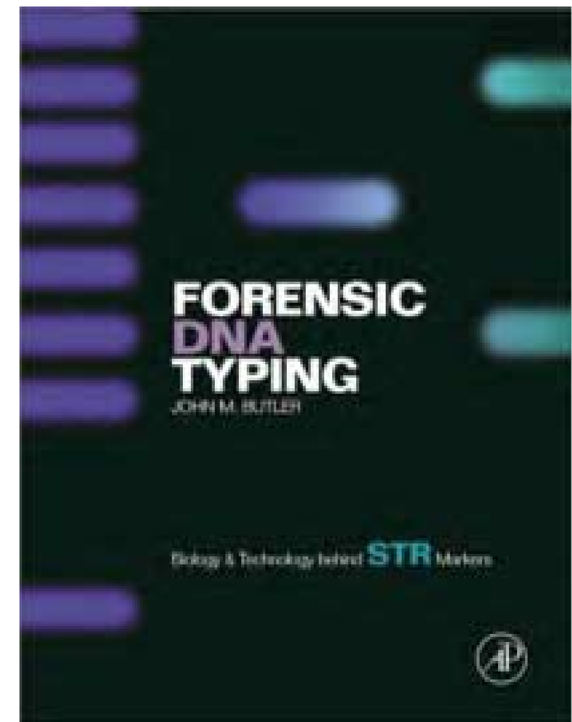
Identifiler Data from a Good Capillary in 3100 Array



Biological "Artifacts" of STR Markers

- Stutter Products
- Non-template nucleotide addition
- Microvariants
- Null alleles
- Mutations

Chapter 6 covers these topics in detail



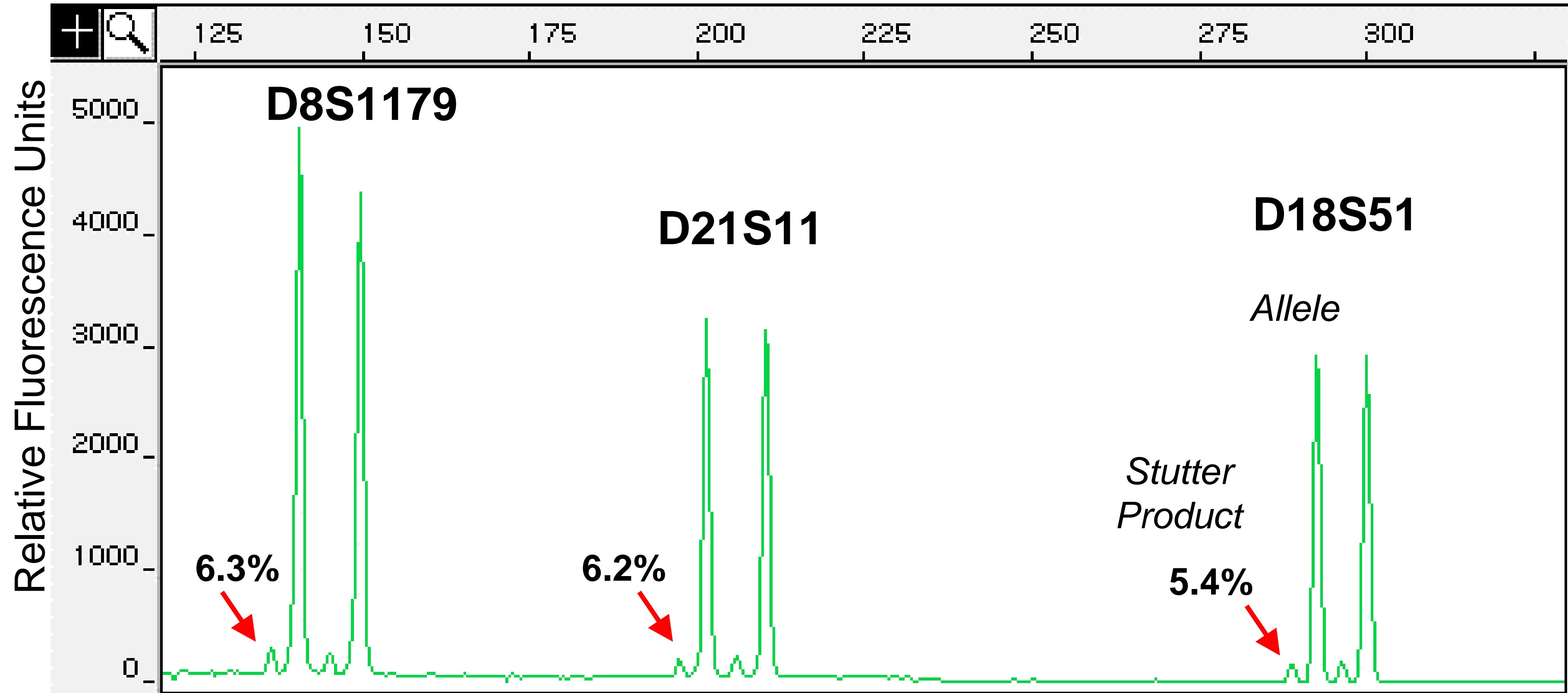
Stutter Products

- Peaks that show up primarily one repeat less than the true allele as a result of strand slippage during DNA synthesis
- Stutter is less pronounced with larger repeat unit sizes

  (dinucleotides > tri- > tetra- > penta-)
- Longer repeat regions generate more stutter
- Each successive stutter product is less intense

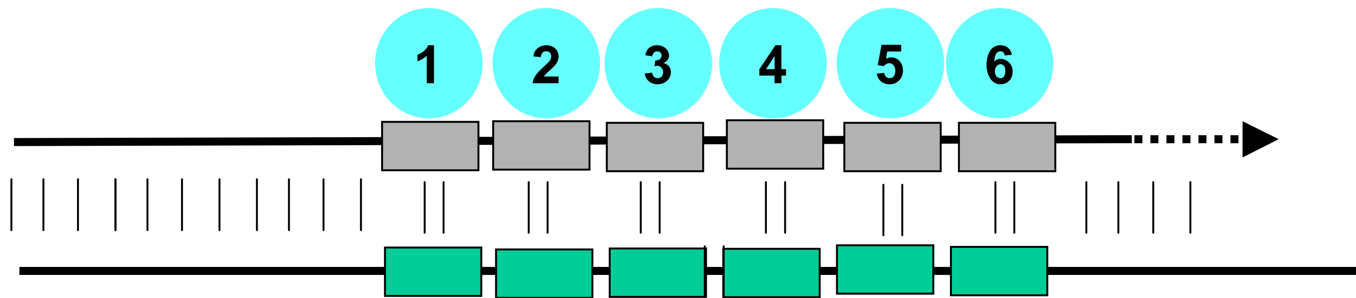
  (allele > repeat-1 > repeat-2)
- Stutter peaks make mixture analysis more difficult

STR Alleles with Stutter Products

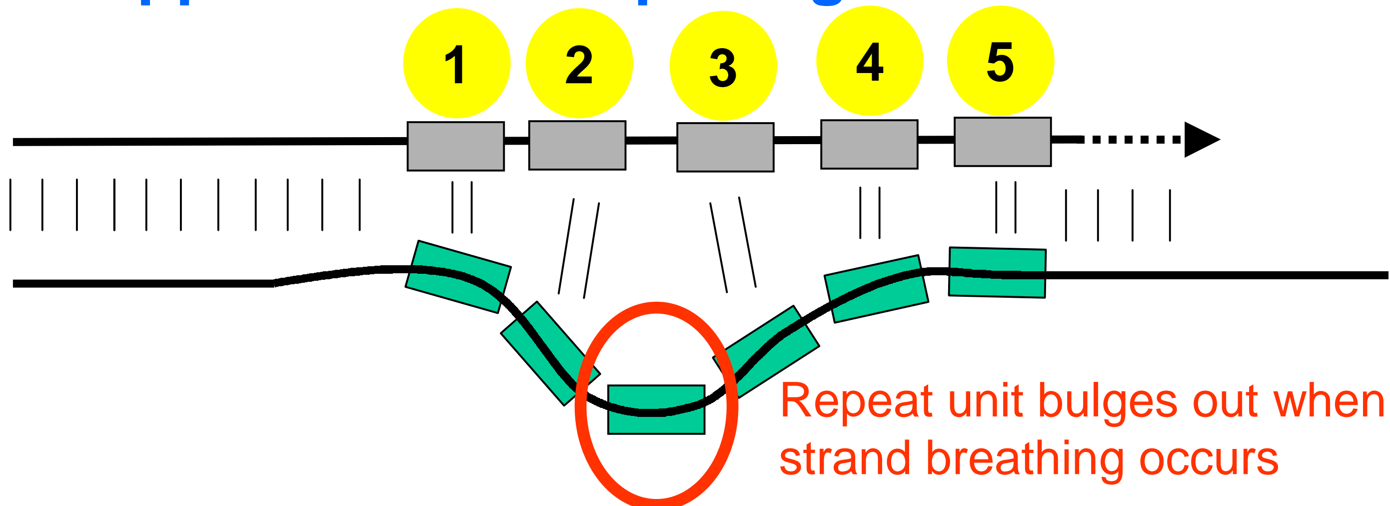


Schematic of Stutter Product Formation Process

Normal STR Allele Replication



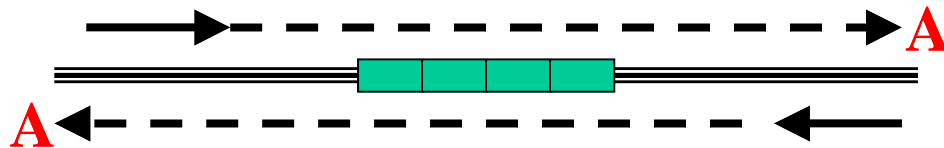
Slipped Strand Mispairing Model



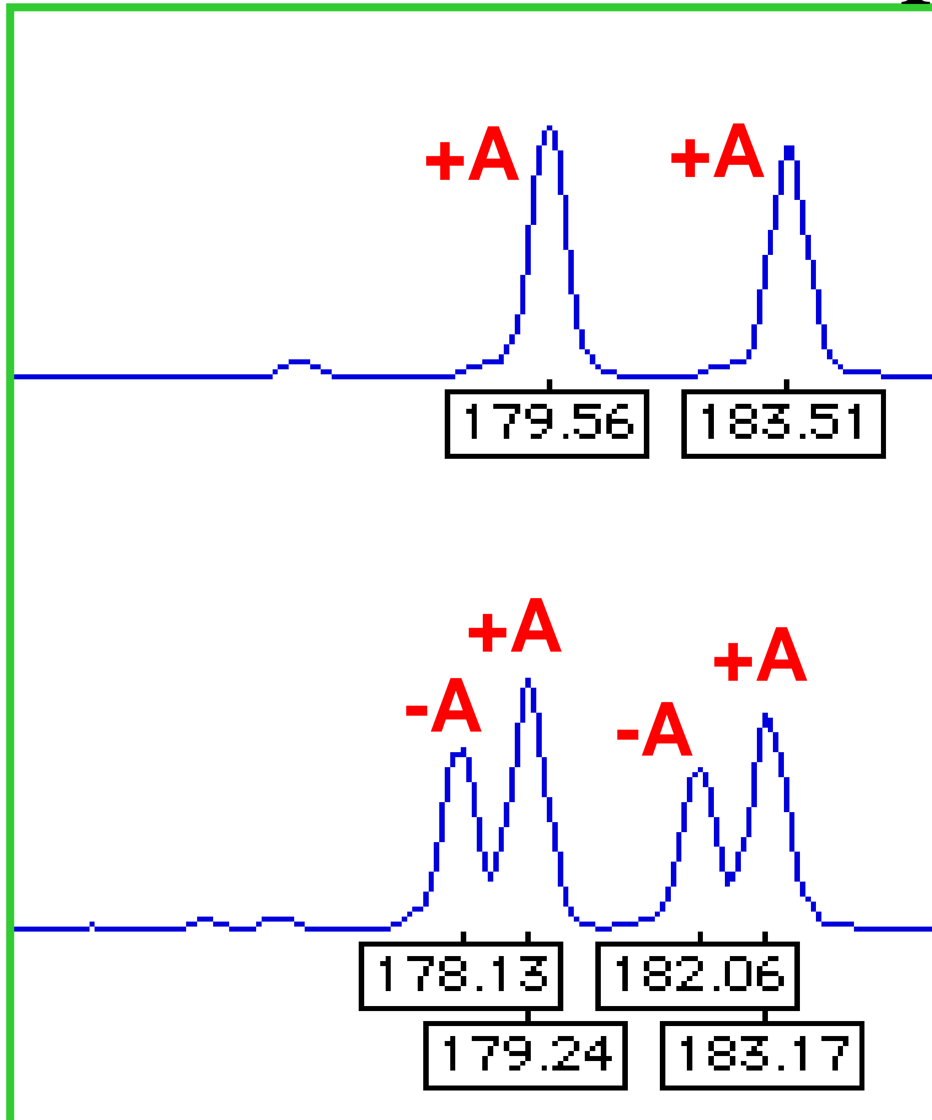
Walsh *et al* (1996) *Nucleic Acids Res.* 24: 2807-2812

Non-template Addition

- Taq polymerase will often add an extra nucleotide to the end of a PCR product; most often an "A"
- Dependent on 5'-end of the reverse primer
- Can be enhanced with extension soak at the end of the PCR cycle (e.g., 15-45 min @ 60 or 72 ^{o}C)
- Can be reduced with new polymerase
- Best if there is NOT a mixture of "+/- A" peaks



Impact of the 5' nucleotide on Non-Template Addition



5'-ACAAG…

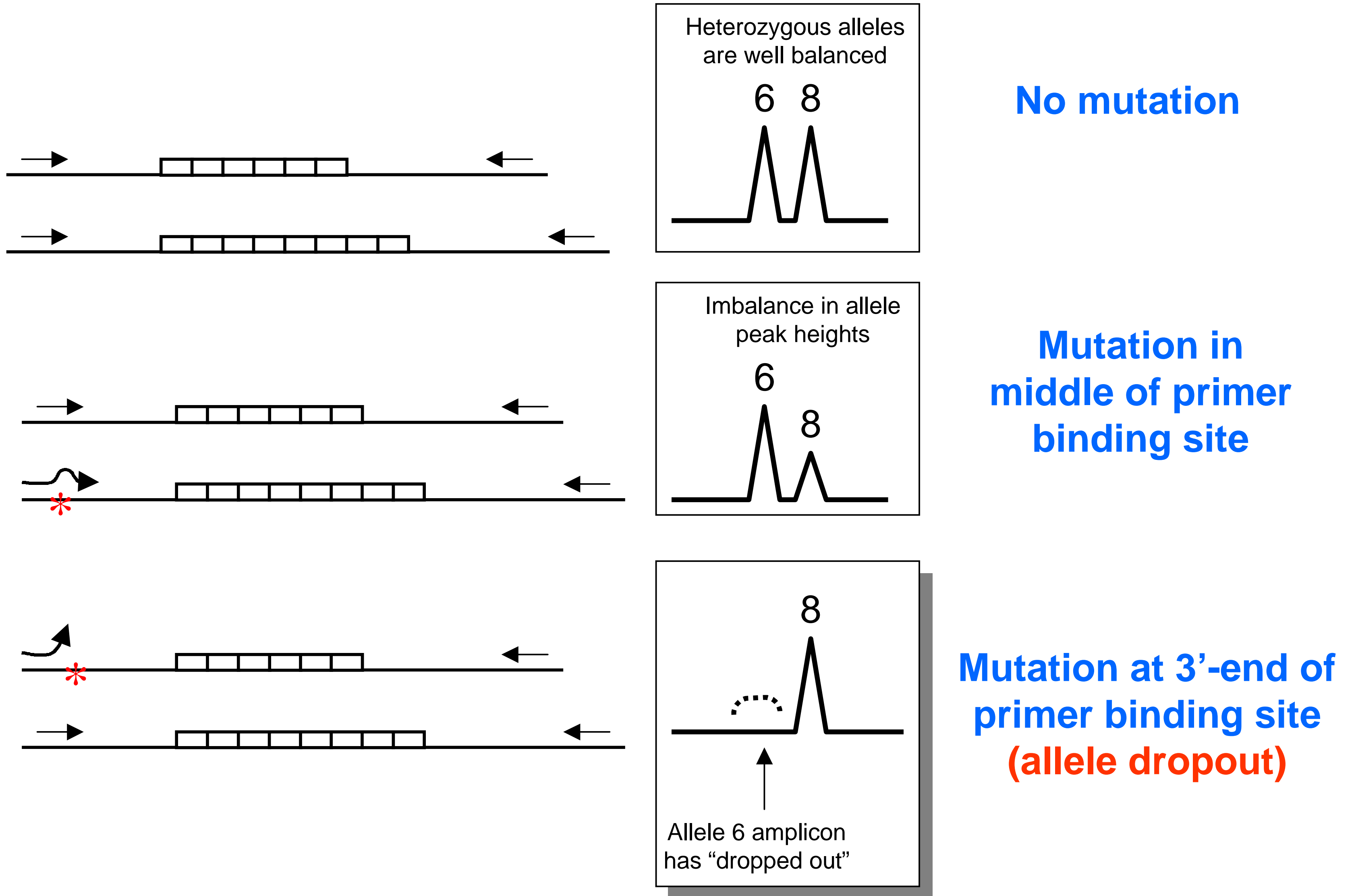
5'-CCAAG…

Last Base for Primer Opposite Dye Label

Null Alleles

- Allele is present in the DNA sample but fails to be amplified due to a nucleotide change in a primer binding site
- Allele dropout is a problem because a heterozygous sample appears falsely as a homozygote
- Two PCR primer sets can yield different results on samples originating from the same source
- This phenomenon impacts DNA databases
- Large concordance studies are typically performed prior to use of new STR kits

Impact of DNA Sequence Variation in the PCR Primer Binding Site



Apparent Null Alleles Observed During Concordance Studies

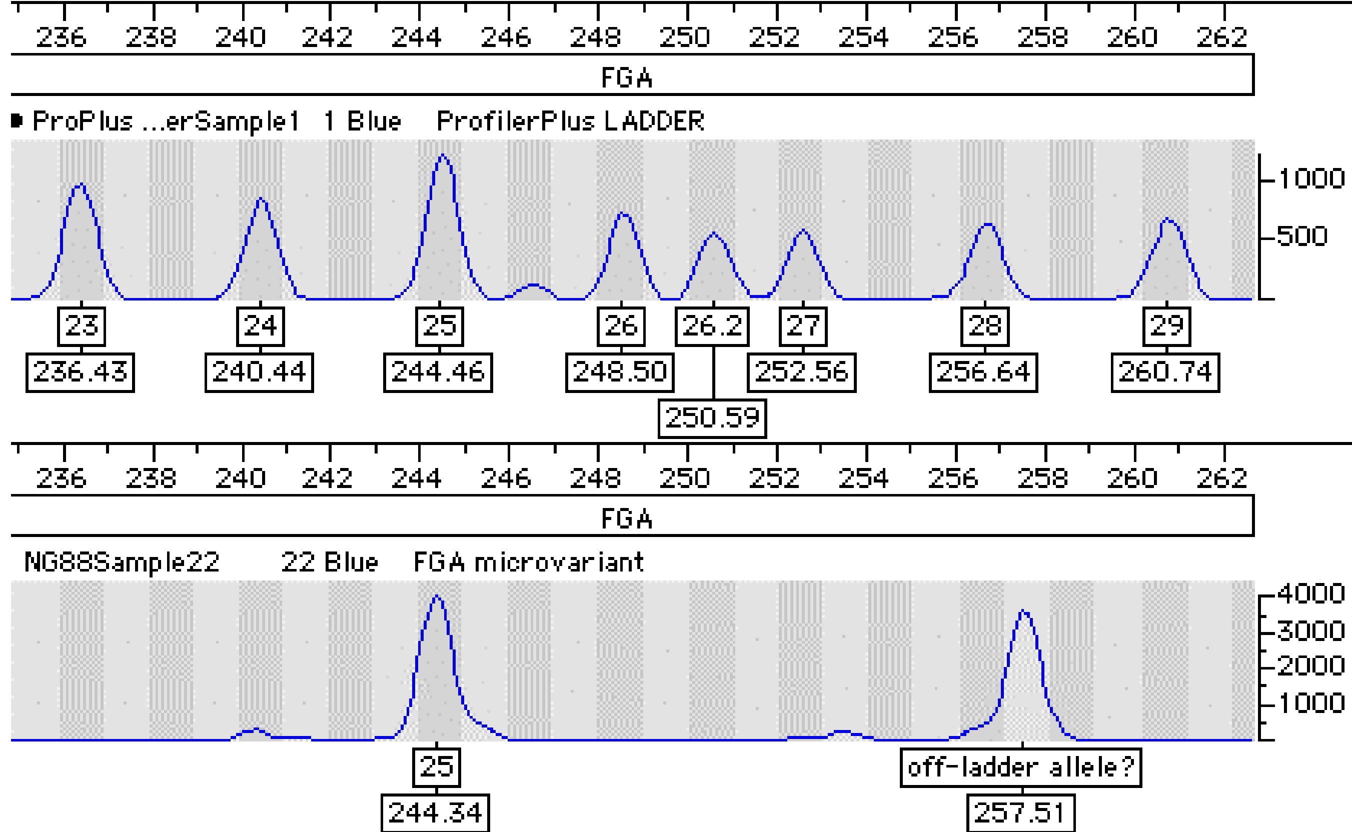
7/13 CODIS loci affected so far

Locus	Kits Compared	Results	Reference
D13	PP1.1 vs PP16 vs ProPlus	Loss of alleles 9,10, and 11 with PP1.1; fine with PP16 and ProPlus	Promega meeting Oct 2000
D13	PP1.1	Reported 4 bp deletion in 3' flanking region while sequencing a rare allele 7 from 2 Asians	Promega meeting Oct 2000 (P#23)
D16	PP1.1 vs PP16 vs COfiler	Loss of alleles with PP1.1; fine with PP16 and COfiler	Promega meeting Oct 2000
D8	PP16 vs. ProPlus	Loss of allele 16 with ProPlus; fine with PP16	Promega meeting Oct 2000
D8	PP16 vs SGM Plus	Loss of allele 15 with SGM Plus; fine with PP16	Promega meeting Oct 2000
VWA	PP1.1 vs. Profiler	Loss of allele 19 in Profiler; fine with PP1.1	Kline 1998
VWA	PP16 vs ProPlus	Weak amp of allele 19 with ProPlus; fine with PP16	Promega meeting Oct 2000 (P#101)
FGA	PP16 vs ProPlus	Loss of allele 22 with ProPlus; fine with PP16	Promega meeting Oct 2000
FGA	PP16 vs ProPlus	Weak amp on allele 21 with ProPlus; fine with PP16	Promega meeting Oct 2000 (P#101)
FGA	SGM vs SGM Plus	Loss of allele 26 with SGM Plus; weak amp of same allele with SGM	Cotton 2000
CSF	PP16 vs COfiler	Weak amp on allele 14 with COfiler; fine with PP16	Promega meeting Oct 2000
CSF	PP16 vs Profiler	Weak amp on allele 8 with PP16; fine with Profiler	Promega meeting Oct 2000
TPOX	PP16 vs Profiler	Weak amp on allele 9 with PP16; fine with Profiler	Promega meeting Oct 2000

Microvariants

- Defined as alleles that are not exact multiples of the basic repeat motif or sequence variants of the repeat motif or both
- May exist as insertion, deletion, or base change
- Sequence variation can occur within repeat, in the flanking region, or in a primer binding site
- Can cause PCR failure due to polymorphism in the primer site -- “null alleles”

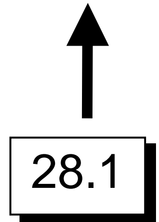
Detection of a Microvariant Allele at the STR locus FGA



$\delta_1 = S_{25}\text{-}L_{25} = 244.34 - 244.46 = -0.12$ bp

$\delta_2 = S_{OL} - L_{28} = 257.51\text{-}256.64 = +0.87$ bp

$c = |\delta_1 - \delta_2| = |-0.12\text{-}0.87| = 0.99$ bp



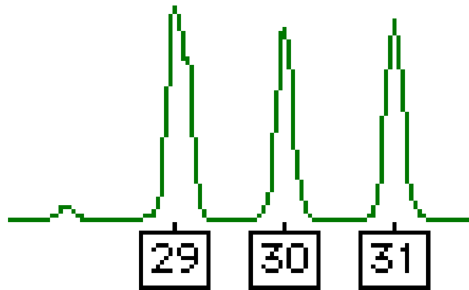
Caution with Sizing Extreme "Off-Ladder" Alleles

FGA ladder allele 30	Unknown FGA allele	Δ bp	Δ repeat	
310 Data				
264.79	330.60	65.81	16.45	(46.2)
264.96	330.63	65.67	16.42	(46.2)
264.66	330.50	65.84	16.46	(46.2)
377 Data				
267.88	329.44	61.76	15.44	(45.2)
267.13	329.89	61.76	15.44	(45.2)
267.56	329.23	61.67	15.41	(45.2)

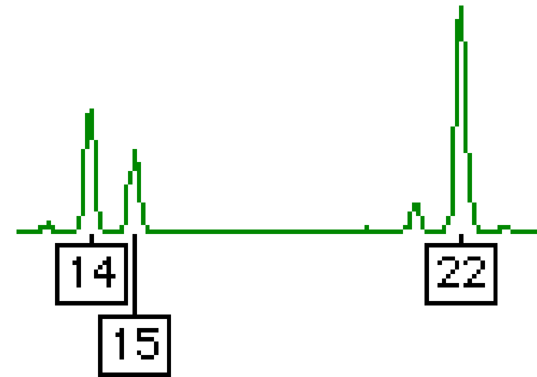
Data courtesy of Melissa Fiebelkorn (Maine State Police Crime Lab)

Three-Peak Patterns

D21S11



D18S51



"Type 1"

Balanced peak heights

Most common in TPOX and D21S11

"Type 2"

Sum of heights of two of the peaks is equal to the third

Most common in D18S51 and …..

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

Database of Variant Alleles

[GATA][GATA][GATA][-ATA][GATA]

D7S820 Variants

150 New Variants

15 total D7S820 variants

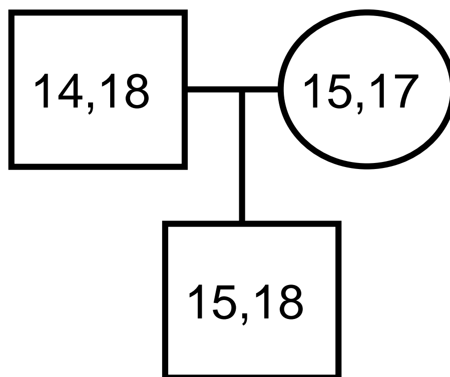
AND 33 unique 3-banded patterns

Allele Designation	Allele Size	Instrument	Amp Kit*	Contributor	Verification/Conformation Method(s)	Notes	Frequency
5	255.55	ABI 377	PS, CO	Nicole Swinton	Re-extracted and Reamplified		
6.3	262.47	ABI 377	PS, CO	Shantel Kaster	Re-amplified	Convicted offender known	1 in 4500
7.3	265.15	ABI 310	PS, CO	Henry Hollyday	Reamplified with two different kits		
7.3 [2]	266.36	ABI 377	PS	Chad Hainley	Reamplified	Convicted offender known	1
8.1	268.27	ABI 377	PS, CO	Nicole Swinton	Re-extracted and Reamplified		

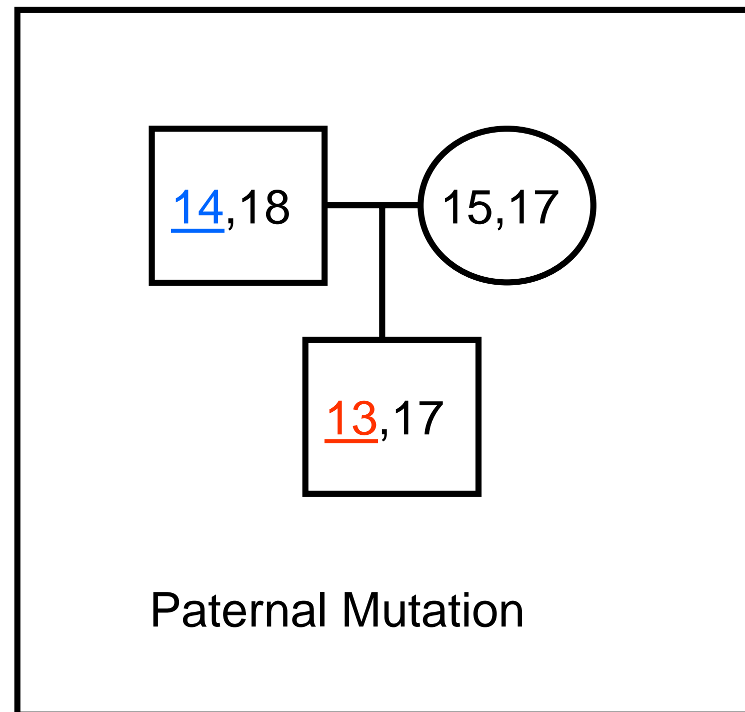
Number of variants reported *(as of Sept 2001)*

D5S818: 3
CSF1PO: 9
D3S1358: 13
D7S820: 15
D8S1179: 2
D13S317: 6
D16S539: 10
D18S51: 20
D21S11: 15
FGA: **45**
TH01: 3
TPOX: 5
VWA: 4

Mutation Observed in Family Trio



Normal Transmission of Alleles
(No Mutation)



Measured Mutation Rates

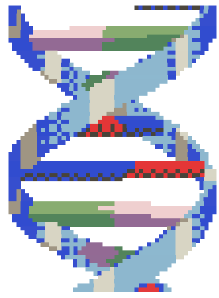
STR Locus	Maternal Meioses (%)	Paternal Meioses (%)	Null Alleles (%)	Multi-Banded (%)
CSF1PO	14/47843 (0.03)	311/243124 (0.13)	2/42020 (<0.01)	*None reported*
FGA	7/8253 (0.01)	555/189973 (0.29)	2/1104 (0.18)	*None reported*
TH01	5/42100 (0.01)	12/74426 (0.02)	2/7983 (0.03)	0/2646 (<0.040)
TPOX	2/28766 (0.01)	10/45374 (0.02)	11/43704 (0.03)	13/42020 (0.03)
VWA	20/58839 (0.03)	851/250131 (0.34)	7/42220 (0.02)	1/6581 (0.02)
D3S1358	0/4889 (<0.02)	9/8029(0.11)	*None reported*	*None reported*
D5S818	22/60907 (0.04)	194/130833 (0.15)	3/74922 (<0.01)	*None reported*
D7S820	14/50827 (0.03)	193/131880 (0.15)	1/42020 (<0.01)	1/406 (0.25)
D8S1179	5/6672 (0.07)	29/10952 (0.26)	*None reported*	*None reported*
D13S317	33/59500 (0.06)	106/69598 (0.15)	52/62344 (0.08)	*None reported*
D16S539	12/42648 (0.03)	40/48760 (0.08)	3/52959 (<0.01)	0/1165 (<0.09)
D18S51	8/8827 (0.09)	29/9567 (0.30)	*None reported*	*None reported*
D21S11	12/6754 (0.18)	17/6980 (0.24)	1/203 (0.49)	*None reported*

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

*Data used with permission from American Association of Blood Banks (AABB) 1999 Annual Report.

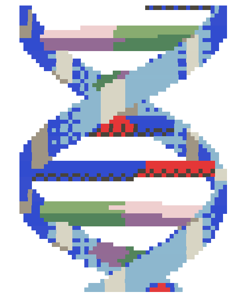
Summary of STR Mutations

- Mutations happen and need to be considered
- Usually 1 in ~1000 meioses
- Paternal normally higher than maternal
- VWA, FGA, and D18S51 have highest levels
- TH01, TPOX, and D16S539 have lowest levels



STRBase

Short Tandem Repeat DNA Internet Database



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

General Information

- Intro to STRs (downloadable PowerPoint)
- STR Fact Sheets
- Sequence Information
- Multiplex STR Kits
- Variant Allele Reports

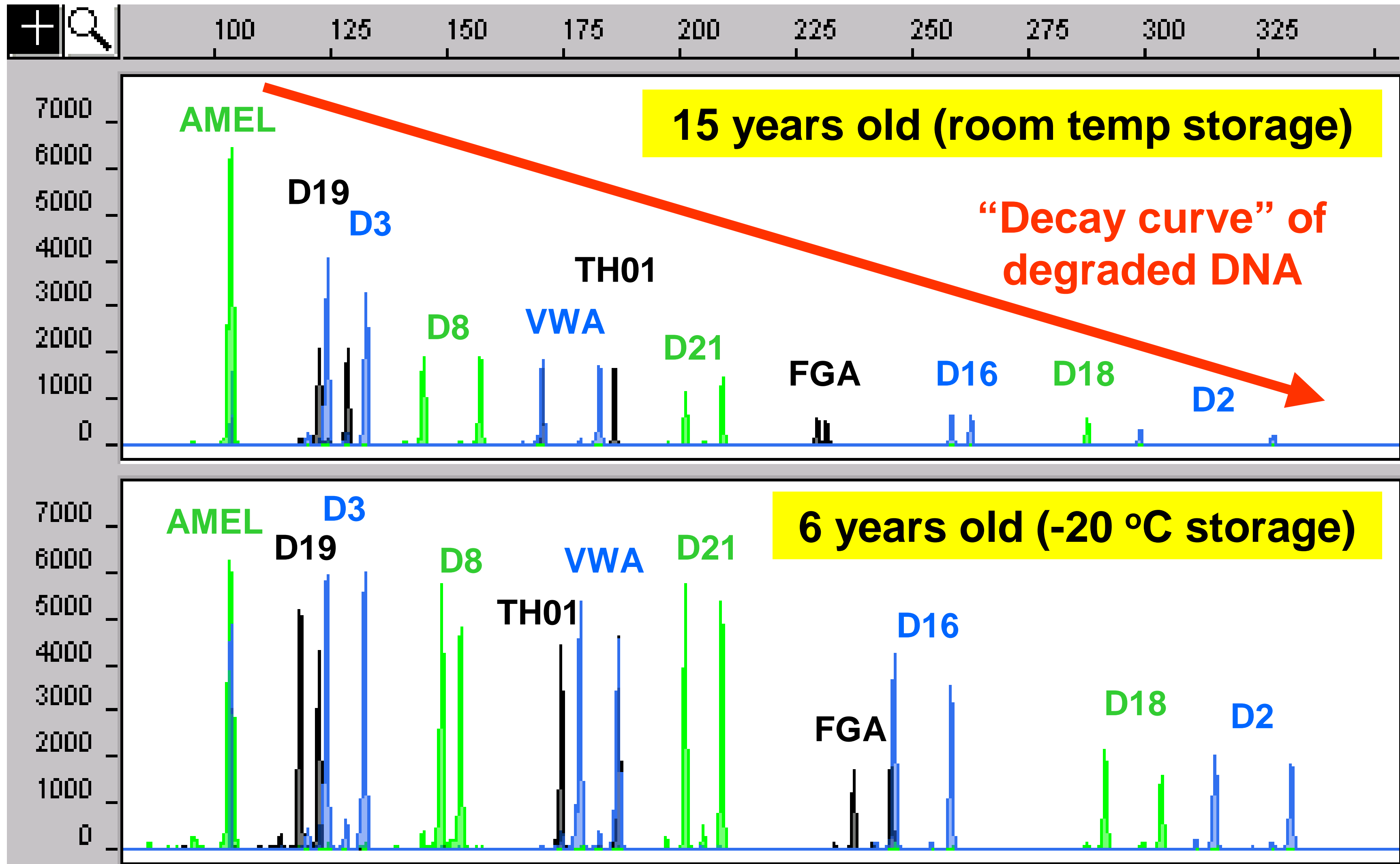
Forensic Interest Data

- FBI CODIS Core Loci
- DAB Standards
- NIST SRM 2391
- Published PCR Primers
- Y-Chromosome STRs
- Population Data
- Validation Studies

Supplemental Info

- Reference List **1479**
- Technology Review
- Addresses for Scientists
- Links to Other Web Sites

Degraded DNA Results



Results with SGM Plus STR kit (Applied Biosystems)

Sample Mixture Example