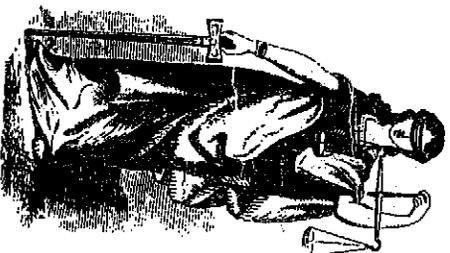


**CONTINUING LEGAL EDUCATION
FALL 2009**

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Forensic DNA Analysis and the Defense Bar

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INSIDE FORENSIC SCIENCE SERIES

FORENSIC DNA ANALYSIS

by

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Interpretation of Mixtures of DNA

Evidence may sometimes consist of a mixture of contributors making the observed pattern more complex. Interpretation of mixtures of DNA can sometimes be confusing. Guidelines need to be established and followed for consistent conclusions. These mixtures may consist of biological material from victim(s) and assailant(s) or they may consist of a single individual combined with one or more contaminating sources. If an STR analysis includes even one heterozygous locus that consists of more than two peaks, the sample is most likely a mixture. However, the overall pattern or genetic profile should be carefully examined before a conclusion is reached that a mixture exists. One should generally not look at only a single locus to form an opinion. A locus that appears to be a heterozygous genotype (i.e. TPOX – 7,12) could be composed of a mixture of two individuals each of whom is a homozygote at this locus (7,7 and 12,12). In this case the peak heights may be the same or different depending on how much of each component is present in the mixture. When examining a single locus, the analyst should consider if it is possible that the sample consists of a sole source, a mixture of individuals who are both homozygous (7,7 and 12,12), heterozygous (7,12 and 7,12) or where one is homozygous (7,7) and the second is heterozygous (7,12). Alternately the homozygous genotype could be 12,12 and the heterozygous genotype could be 7,12. If equal amounts of DNA from each individual are present, then in the first case (7,7 + 7,12) the 7 allele (peak) will have a peak height 3 times as high as the 12, whereas in the second case (7,12 + 12,12) the 12 allele will have a peak height 3 times the height of the 7 peak as shown below:

Figure 5 -

The multiplex kits are also designed in such a way that if all DNA samples are quantified to be within a specific range (approx. 1-5 ng), the resulting peak heights at all loci will be within 25-30% of each other (experimentally determined). If alleles are markedly different in peak heights, a mixture of template DNAs should be considered. When a mixture of DNA components is combined in different quantities, results can be observed with variable peak heights requiring further interpretation.

Degraded DNA

Where DNA within evidentiary samples has become degraded, the larger alleles within loci may be decreased in peak height or missing depending on the amount of DNA breakdown. Thus the larger allele of a heterozygous genotype may be missing and the locus can appear as homozygous. In addition, all alleles for the larger STR loci may be missing. This is something that should be considered in samples that are old or have been exposed to environmental factors such as sunlight, soil, bacteria or fungi.

DNA degradation refers to the breakdown of relatively large fragments of DNA into smaller fragments. When this breakdown becomes advanced, the target DNA sequences for the PCR amplification reaction (which must contain both primer annealing sites) are also broken down. Thus at any locus (e.g. D18S51 with alleles in the range of 274–342bp), it is more likely that the larger alleles will be lost in a partially degraded sample.

Partial degradation can be inferred when the AmpF/STR Profiler Plus™ Kit is used (to amplify the 9 STR loci and amelogenin) and alleles of the smaller loci D3S1358, D8S1179, and D5S818 are seen, but alleles of the larger loci D18 and D7 are not seen.

Artifacts in Observed Results

The analyst should examine the results carefully to verify that all observed peaks are real and not due to some artifact produced during the amplification, fragment separation or detection processes. Some observed alleles are uncommon and are therefore not included in the allelic ladder. Such alleles when observed require authentication. Some common artifacts and their explanations are described below.

1. Stutter:

If examination of the genetic profile indicates a sole contributor, and the printout indicates a small peak either -4 bp or +4 bp adjacent to a larger peak. The “-4 bp” stutter is much more common and will sometimes be labeled by the software as an authentic peak. The “+4 bp” stutter is extremely rare and has to be interpreted carefully since it may indicate the presence of a mixed specimen. Some loci are more prone to amplify with stutter (i.e. VWA) than others. Experimentation shows that on average, stutter peak height ranges from approximately 2 to 14% of the adjacent peak. Such small -4 bp peaks are not reported by the software as alleles.

2. Pull Up Peaks

“Pull-ups” of peaks in any color caused by a very high peak of another color in the same injection. Pull-ups are caused by the inability of the matrix file of the software to remove all overlap, i.e., green light components originating from a strong blue signal.

3. Split Peaks

These are sometimes referred to as “N” bands where the main allele appears as a split peak. “N” bands are caused by incomplete extra Adenine addition (a PCR artifact) and are characterized by either a jagged edge on the left side of the peak or a complete split on the top level. The split peak is caused by two fragments differing in size by only a single Adenine base.

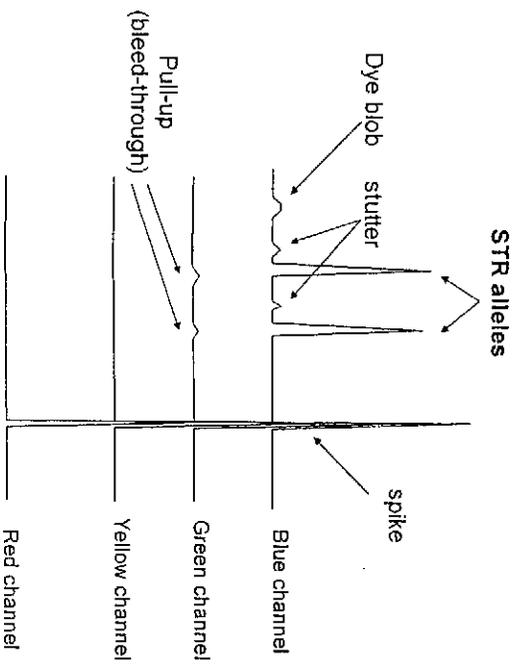
4. Shoulder Peaks

Shoulder peaks approximately 1-4 bp smaller or bigger than the main allele. Shoulder peaks are most often apparent on the right side of a large peak especially if the peak shape appears to have a trailing slope.

5. Spikes in printout

Spikes are sharp peaks that do not have the characteristics of normally shaped peaks but rather appear as vertical lines. These spikes may be caused by air bubbles or urea crystals in the capillary. The presence of a spike can sometimes be verified by examining the printout of the calibrator red size standard for the same injection or any of the other colors. Such "spikes" will occur at the same position for all colors.

Deciphering Artifacts from the True Alleles



6. Noisy or Raised Baseline

Sometimes the software will call a peak as an allele when that peak is on top of a raised baseline. A raised baseline can be observed when the polymer within the capillary is dirty or there are capillary problems.

7. Dye Artifact or Dye "Blob"

Dye artifact peak which appears more like a cup shape than a peak. These artifacts (usually blue) appear at a constant position on the printout.

8. Non-Specific Artifacts

Non-specific artifacts. These are artifacts that cannot be described by one of the above explanations. Such peaks cannot be reproduced despite repeated testing.

9. Errors in Interpretation due to Mutation – Null Alleles

In some rare occasions, a heterozygous genotype may appear as homozygous. This can occur when there is a mutation in the primer-binding site of an allele which prevents one of the primers from annealing as it should. It is also possible for a mutation close to the primer binding site to block extension or new DNA during amplification. A comparison between evidence and exemplar (known) samples based on a locus where both samples were amplified with the same primer sequence is no problem. However, if the same locus is amplified using different multiplex systems it is possible to obtain a heterozygous type with one multiplex and an apparent homozygote with the second. This occurs because different multiplex kits may use different primers for the same loci and a mutation may occur in only one of the primer binding sites. In such a case, the heterozygote type should be considered the correct type. For this reason, all samples from the same case should be tested with the same multiplex system.

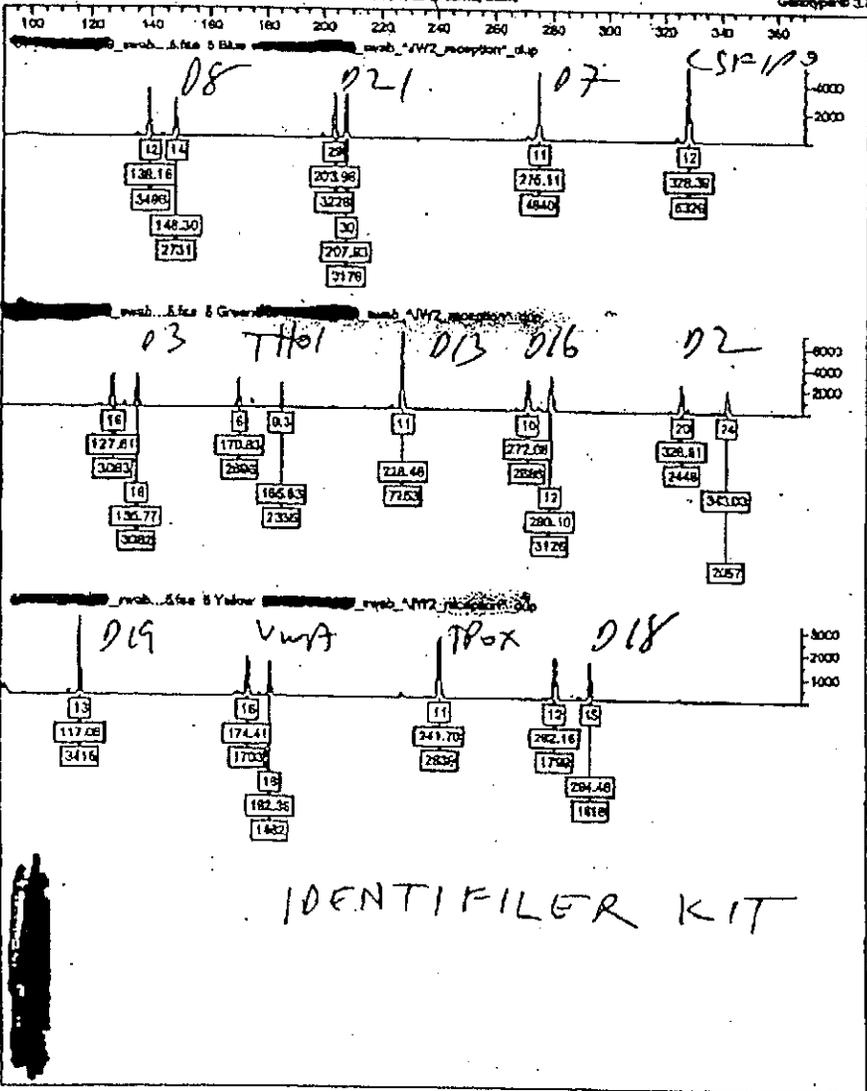
Although the interpretation of mixed samples can be difficult and results from the electrophoresis may include artifacts, careful observation and adherence to the typing protocol

will allow accurate conclusions about a sample's genetic profile. The next chapter reviews the interpretation of a mitochondrial DNA sequencing analysis.

ABI PRISM

Plot - Human07-018D-E.MB.gis
Licensed to some, some

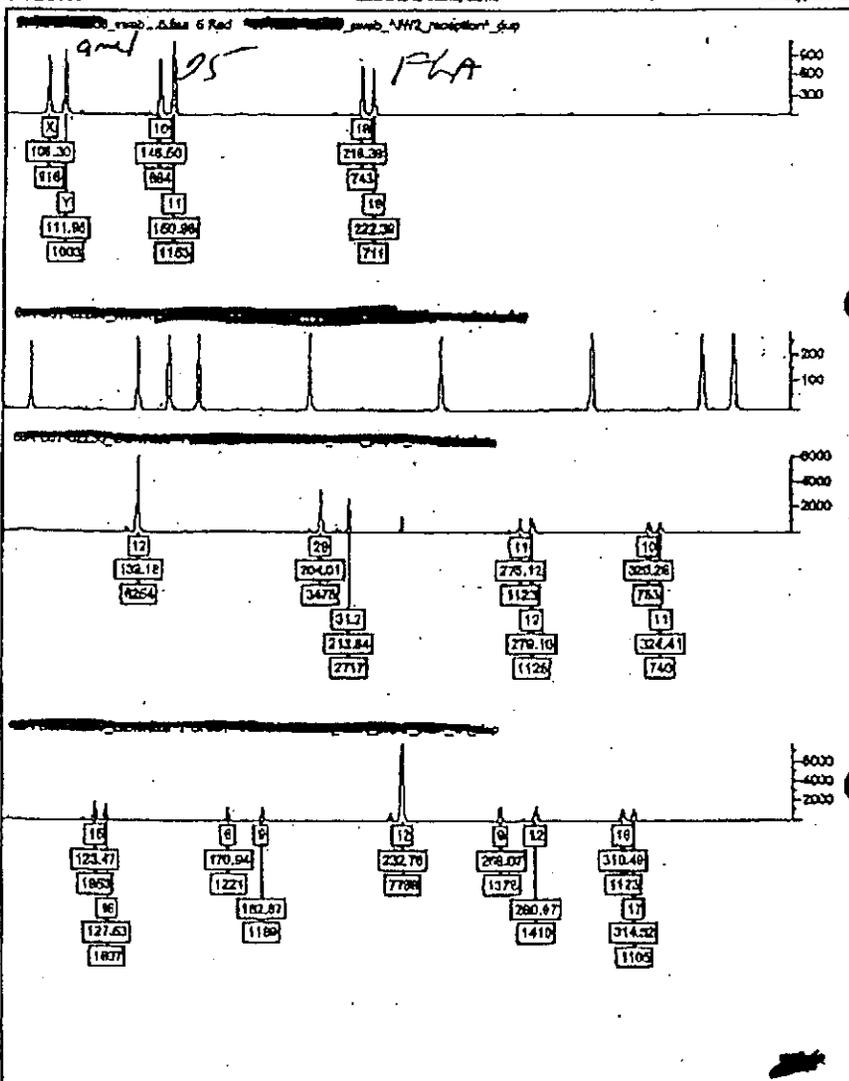
Genotype 3.7

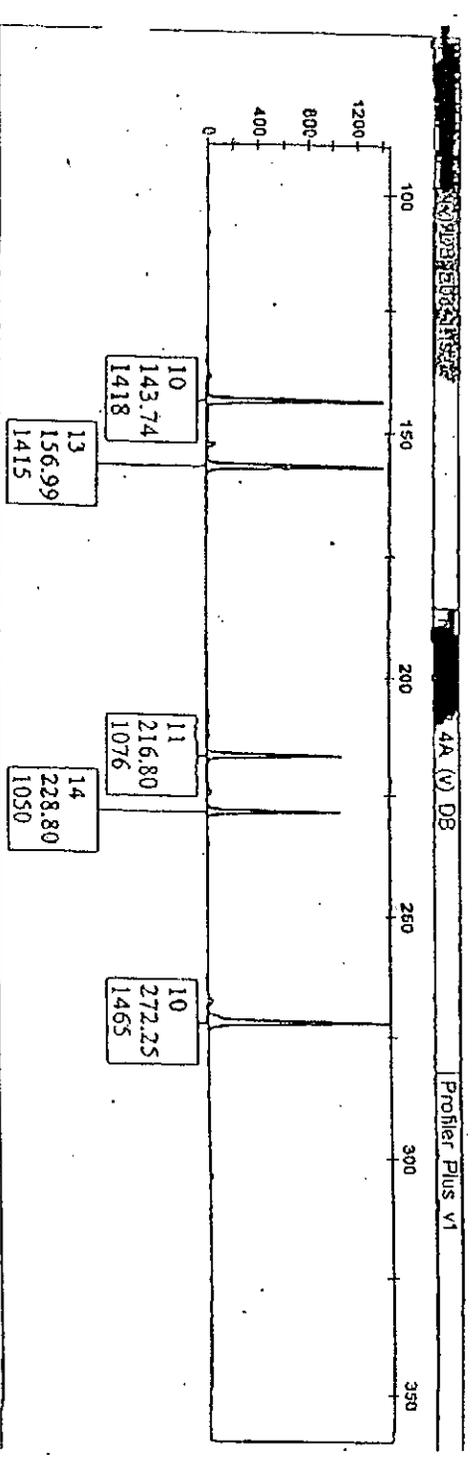
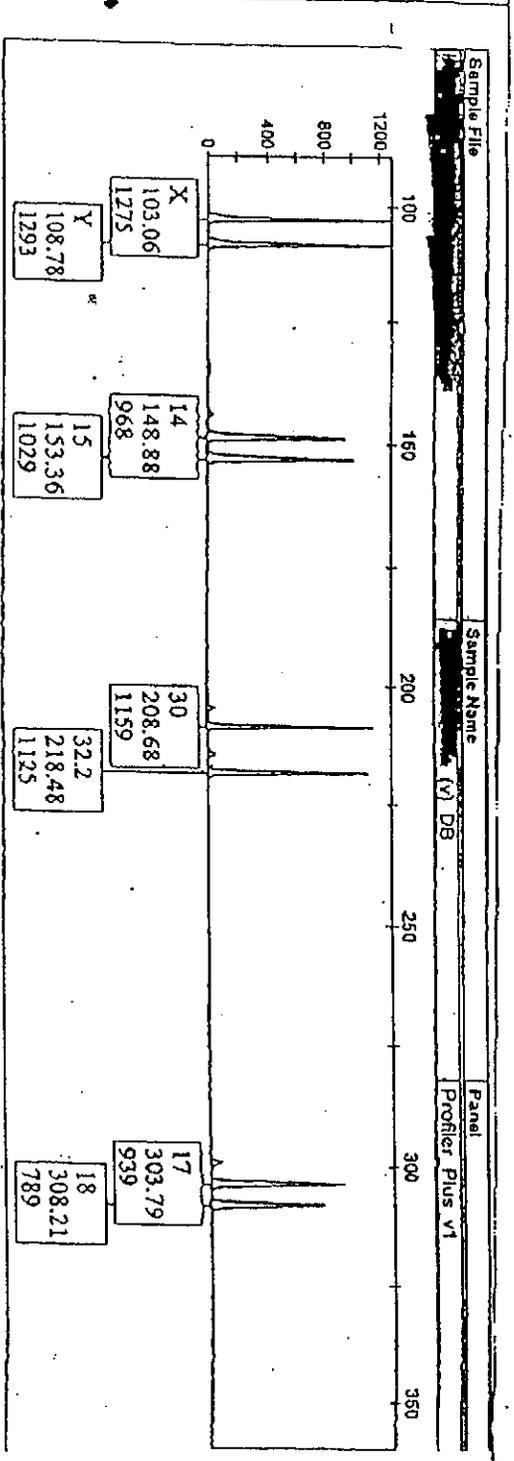
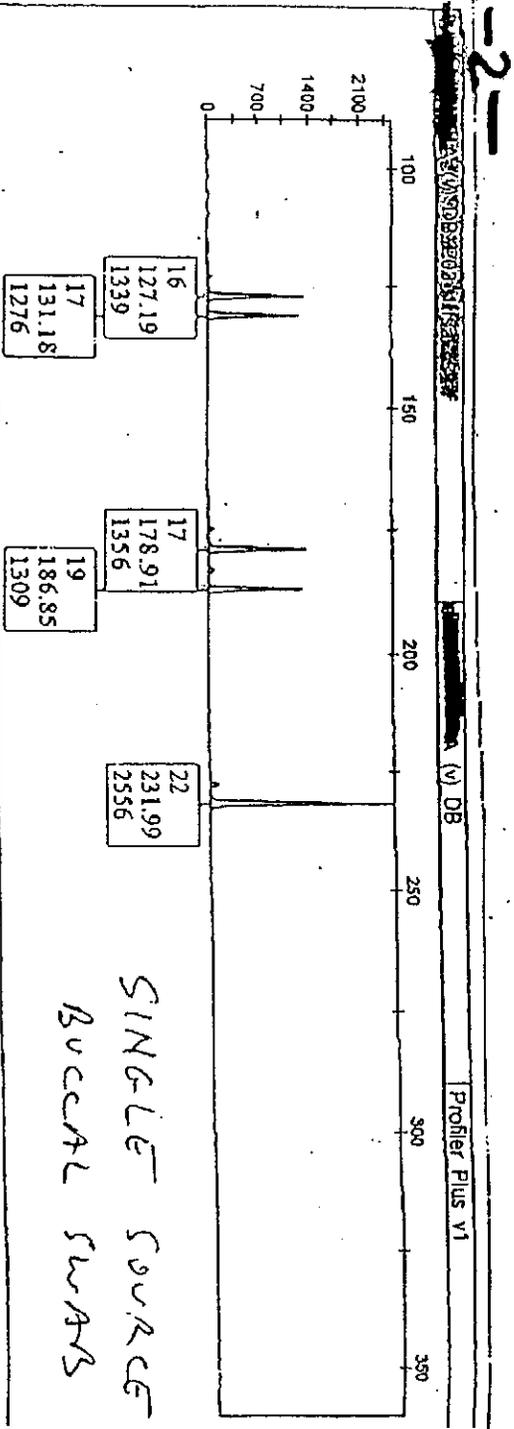


ABI PRISM

Plot - Human07-018D-E.MB.gis
Licensed to some, some

Genotype 3.7





ABI PRISM 3-

Plots - Rudy05-003COC-Imm.gta

Genotype 3.7

4 Yellow Jacket st. 2E EC

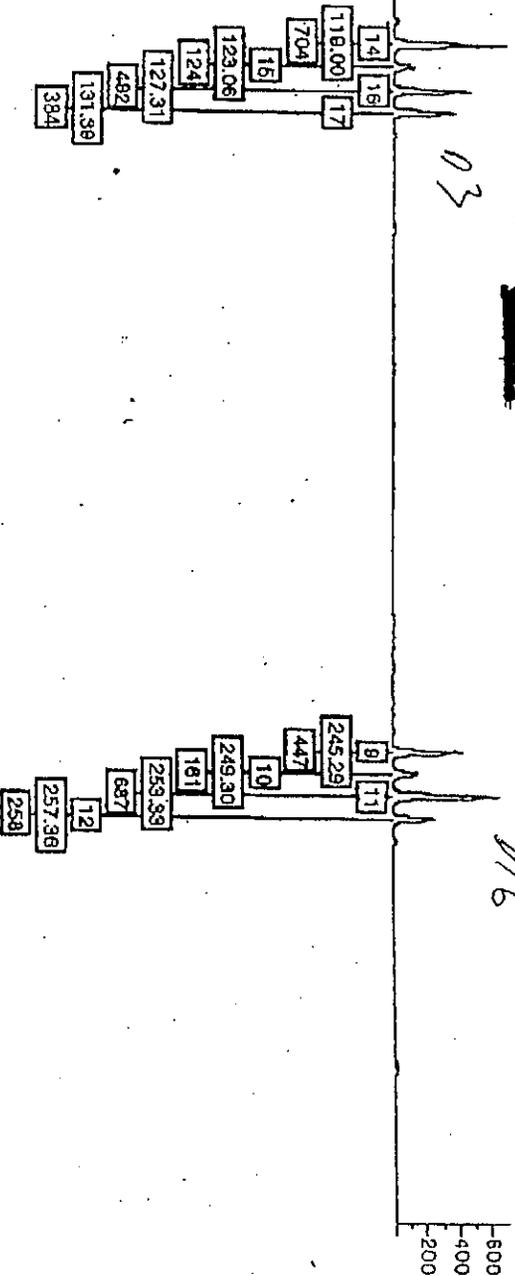
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MIXTURE
INTERPRETATION

6 Blue Handcuff swab 1A m'oon

03

016



ABI PRISM

-4-



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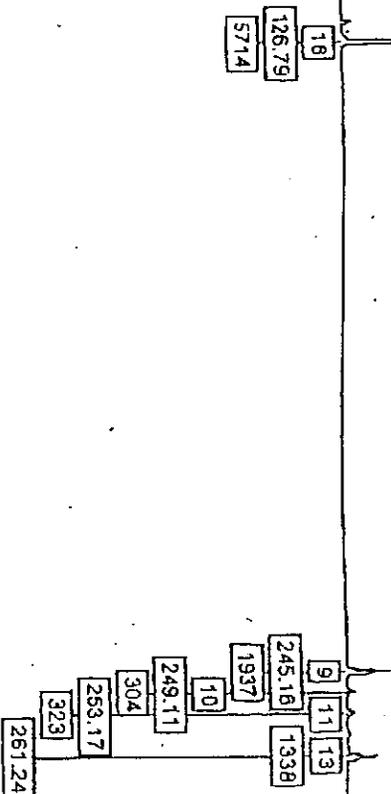


Genotyper® 3.7

B Blue DS1A4 'breast, pelvic, perirectal' SR"

03

016

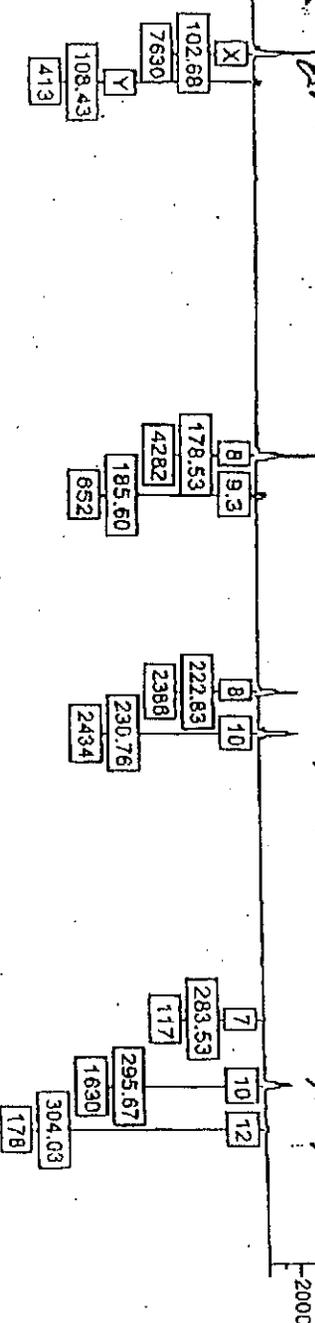


B Green DS1A4 'breast, pelvic, perirectal' SR"

TH 1

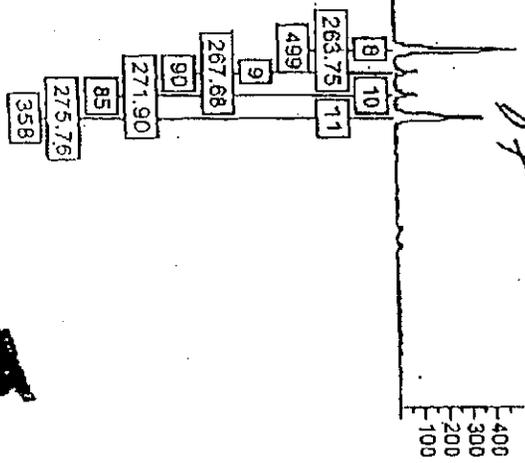
TH 2

CP 1/10



B Yellow DS1A4 'breast, pelvic, perirectal' SR"

07

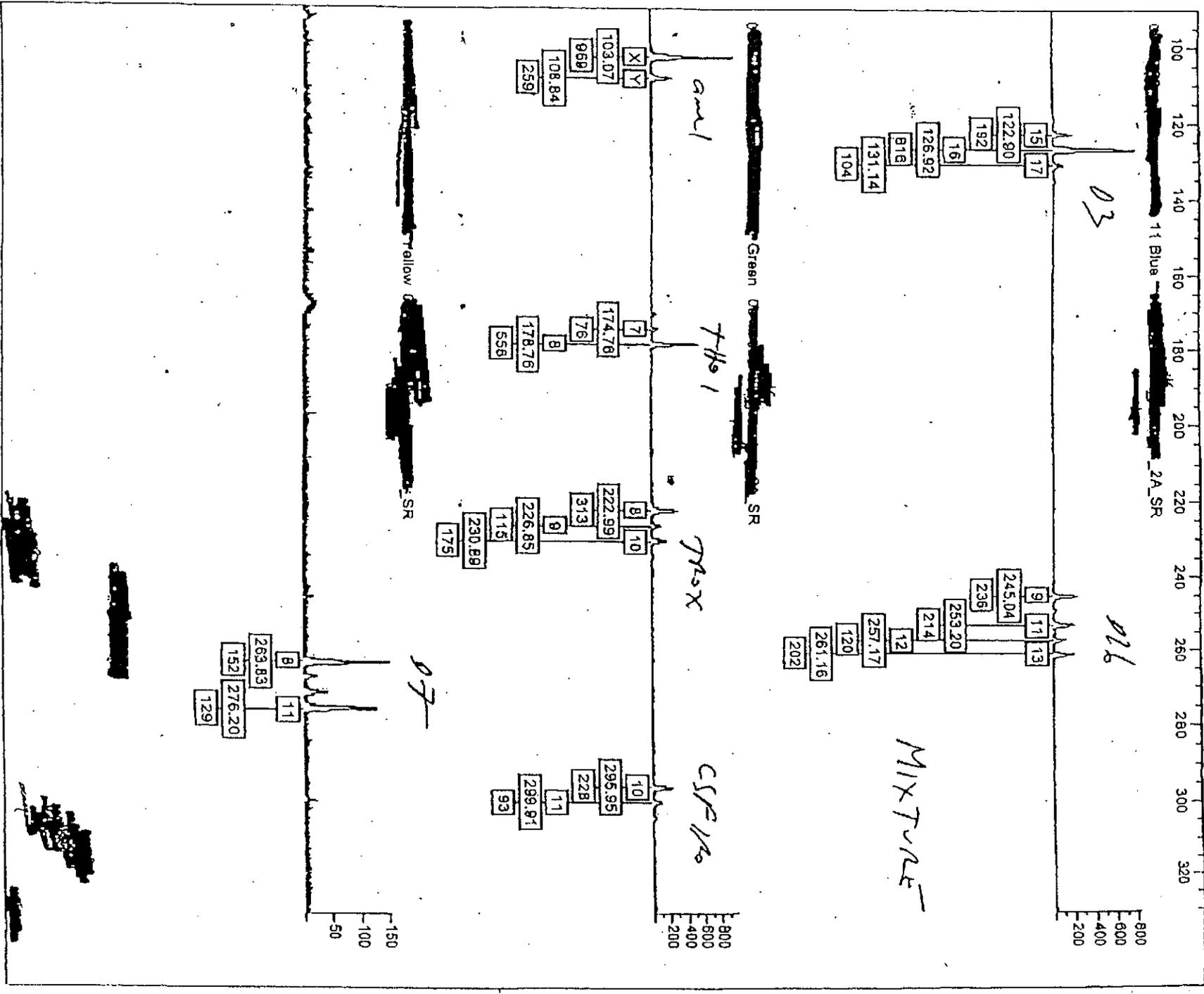


ABI PRISM

-5-

Plots - Rudy07-101CoA 1BL.g1a

GenDyperm 3.7

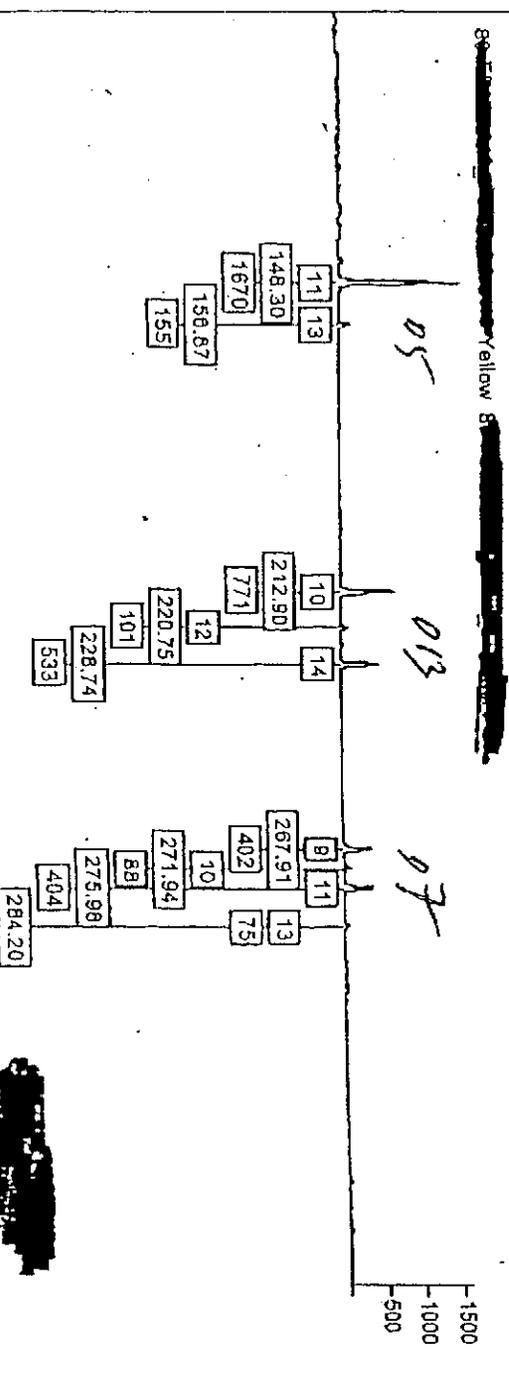
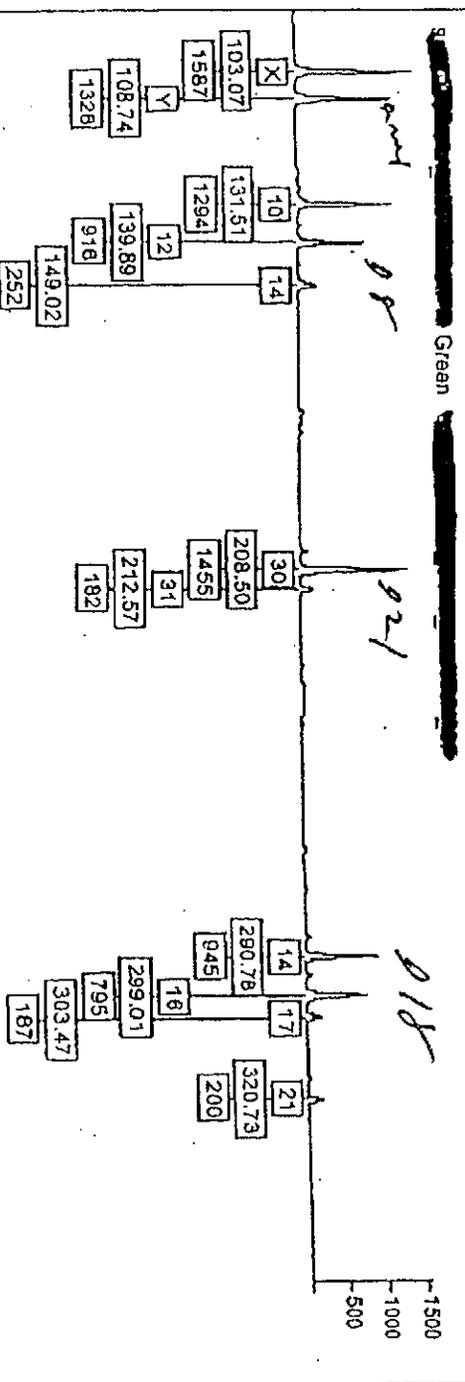
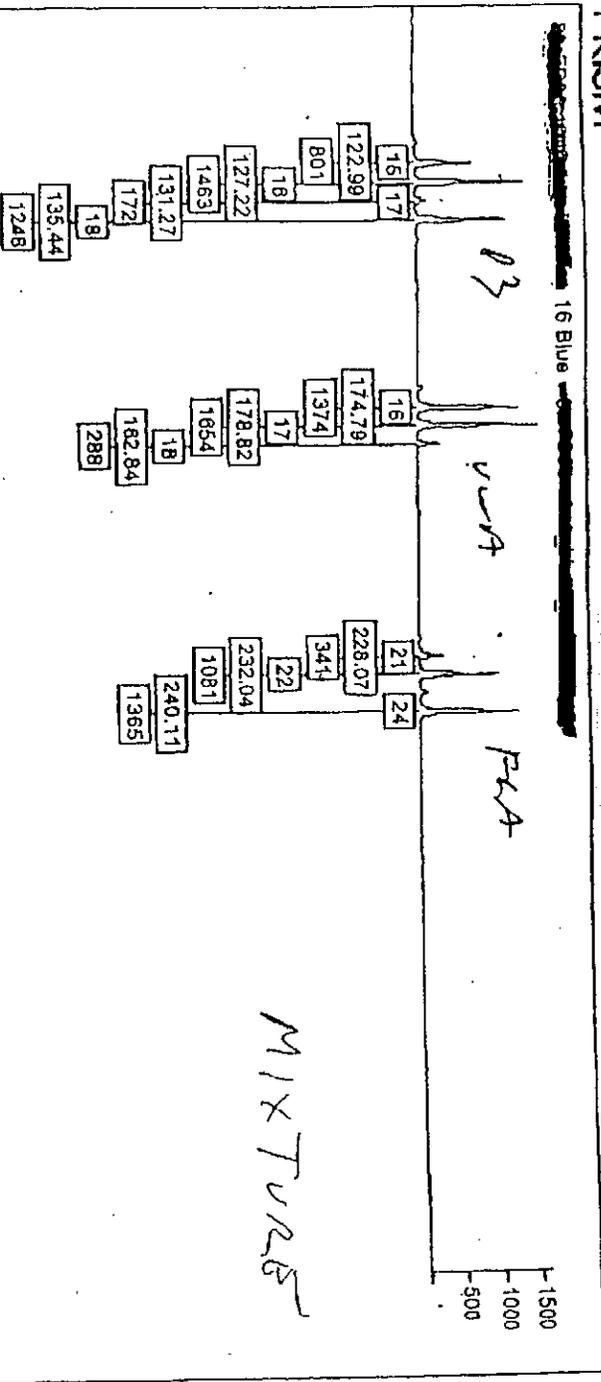


ABI PRISM

-6-

Plots - Rudy07-025Pro KB.gla

Genotype 3.7



ABIPRISM

-8-

GenType03.7

54_F07_STARS_11.1sa

No Size Data

54_F07_STARS_11.1sa

No Size Data

57_A08_STARS_02.1sa

2 Blue Extraction Negative

PROBLEM
NEG CONTROL

90
60
30

13
281.38

15
123.03
18
127.10

57_A08_STARS_02.1sa

2 Green Extraction Negative

100
50

X Y
102.89
108.81

8
222.86

57_A08_STARS_02.1sa

2 Yellow Extraction Negative

No Size Data

NB



-9-

Plate - Stars07-025Y_SCK.gta
Licensed to ocme, ocme

Genotype® 3.7

Yellow

No Size Data

2 Blue

No Size Data

Y STR

4 Yellow

1Y15

0Y13091

0Y538911

13 184.78
517

13 250.73
391

29 368.52
255

4 Blue

SF

0Y13390

25 217.80
489

8 Yellow

SF

14 188.59
699

13 250.46
360

28 368.49
275

6 Blue

23 209.90
755

600
400
200

600
400
200

400
300
200
100

400
300
200
100

2/18
AV
SCT

-10-

