DNA Mixture Analysis:
Principles and Practice of Mixture Interpretation and Statistical Analysis
Using the SWGDAM STR Interpretation Guidelines

Updating Your Protocols:
Lessons Learned…
and still Learning

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Outline

- Validation
- Manual updates and training
- Observations
- Application of new interpretation guidelines and report writing for three case examples
- Improvements

In the beginning…

- PowerPlex®16 Amplification Kit
- Approximately 1ng max in amp reaction
- 310 Genetic Analyzers
- 100 rfu single threshold
- 3, 5 and 10 sec injection parameters
- 70% PHB (peak height balance)

So what's all the buzz?

- Attended the 2008 AAFS Workshop on Mixture Interpretation

- CE User's Meeting hosted by ATF and John Butler was the guest speaker

- CE User's Meeting hosted by MSP with Bruce Heidebrecht's mixture exercises

Our Validation Journey begins

- Replacing our 310s with a 3130 Genetic Analyzer

- Determine our Limit of Detection (Analytical Threshold)

  3 sec injection = 50 rfu
  5 sec injection = 60 rfu
  10 sec injection = 80 rfu

Stochastic Threshold

- Defined as "*peak height below which the second allele of a heterozygous pair may not be detected*"

- 10 individuals, triplicate amps at 0.05ng and 0.075ng

- Evaluate ~400-450 loci / injection parameter

Stochastic Threshold

- 3sec injection = 230 rfu
  - One instance with an allele detected at 305 rfu and the second allele was not detected above the analytical threshold

- 5sec injection = 310 rfu
  - One instance with an allele detected at 464 rfu and the second allele was not detected above the analytical threshold

- 10sec injection = over 600 rfu
  - One instance with an allele detected at 704 rfu and the second allele was not detected above the analytical threshold

Mixture Study

- Ratios examined and 4:1 mass ratio was determined to be the cut-off for determining a distinct major DNA profile for an RMP statistic without a forensically valid assumption

- 60% will the minimum peak height balance for analyzing mixed samples

- All alleles at a locus must be above the stochastic threshold to be considered for a CPI statistic

Variance noted in validation

- Unexplainable variation in rfus were observed during the validation studies

- Coordinated with Applied Biosystems (Tim McMahon) and ran a pooled amplicon on a full tray on our 3130 and on Tim's 3130

- 1.5 fold difference is acceptable within a well (reinjections) and well to well variance may even exceed 1.5 fold difference.

Variance Summary

- 1.5 fold difference is acceptable as long as the variance is not consistently different or a reoccurring issue.

- Capillary to capillary variance should not show more than a 1.5 fold difference.

Manual Updates

- Use your community as a great resource to gather validation studies and manuals
- Insert a flowchart into your interpretation section for a streamlined approach to the new mixture interpretation
- Generate a worksheet to document mixture deconvolution in the case file
- Update report writing

Interpretation section of the manual

- "An overall mass ratio of approximately 4:1 will act as a threshold as to whether a distinct major contributor can be deduced from a mixture of three or more individuals. This mass ratio is calculated by adding the heterozygote peak heights of the major contributor, divided by the total peak heights of all minor contributors."

Interpretation section of the manual

- 4:1 mass ratio is also in place for a 2 person mixture as to whether a distinct major contributor can be deduced unless a forensically valid assumption can be utilized.
- "A forensically valid assumption presumes that this person's DNA is expected to be in a mixture and therefore can be subtracted from the profile."

Interpretation section of the manual

- Examples of types of evidence that qualify for a FVA: swabs from a rape kit, fingernail clippings and scrapings, stains on clothing worn by the person
- Not every possible item of evidence where a FVA may be used is defined and this is addressed in the manual as "…specific case circumstances may be considered for use of the FVA with approval".

Mixture Interpretation Worksheet

Exhibits Locus \		Evidence		Mass Ratio		Peak Height Balance	Statistic
D3S1358							
TH01							
D21S11							
D18S51							
Penta E							
D5S818							
D13S317							
D7S820							
D16S539							
CSF1PO							
Penta D							
Amelogenin							
vWA							
D8S1179							
TPOX							
FGA							

Exhibits Locus \			Evidence		Mass Ratio		Peak Height Balance	Statistic
D3S1358								
TH01								
D21S11								
D18S51								
Penta E								
D5S818								
D13S317								
D7S820								
D16S539								
CSF1PO								
Penta D								
Amelogenin								
vWA								
D8S1179								
TPOX								
FGA								

Enter the profile of an individual (ex: Complainant) if a FVA is being used for the mixture interpretation

Exhibits Locus \		Evidence				Mass Ratio	Peak Height Balance	Statistic
D3S1358								
TH01								
D21S11								
D18S51								
Penta E								
D5S818								
D13S317								
D7S820								
D16S539								
CSF1PO								
Penta D								
Amelogenin								
vWA								
D8S1179								
TPOX								
FGA								

This column will be the deduced major or minor contributor

Report Writing

- If all loci are below the stochastic threshold: "*The DNA profile obtained from the EVIDENCE (ITEM #) does not meet the required interpretation criteria and can only be used for elimination purposes*."

- If an individual cannot be excluded from data below stochastic threshold: "*Due to limited results, INDIVIDUAL can neither be included nor excluded as a possible contributor to the DNA profile obtained from the EVIDENCE (ITEM #).*"

Reporting profiles in a table

- A table is included in the report if a statistic is reported for an item of evidence.

- Use "*" next to any allele below the stochastic threshold and add "* designates allele below stochastic threshold" to the key of the table.

Training for the Unit

- Discuss and review the validation results and summary
- Prepare practice exercises requiring mixture interpretations followed by report writing and statistics if applicable
- Meet again to review the exercises
- Prepare a qualifying test combining the new interpretation guidelines, report writing and statistics.

Additional tips to ease the transition

- Review case files and share with the section regularly
- Meet with investigators to educate them on the results
- Meet with the State's Attorney's Office to have a training session on the new interpretation guidelines

Observations

- Length of time required to complete the mixture worksheets

- Streamlined interpretation for many cases

- It didn't take long to have cases that didn't quite "fit" in the flowchart

Case Example #1

- Sexual Assault
- Two suspects and only one suspect is located
- Analysis conducted on the Complainant's underpants
- No prior consensual partners indicated

Underpants

- A stain was recovered from the right hip area of the underpants.

- Semen identified in the stain.

- The non-sperm fraction matches the Complainant.

- Here is the profile detected from the sperm fraction:

Sperm fraction from underpants
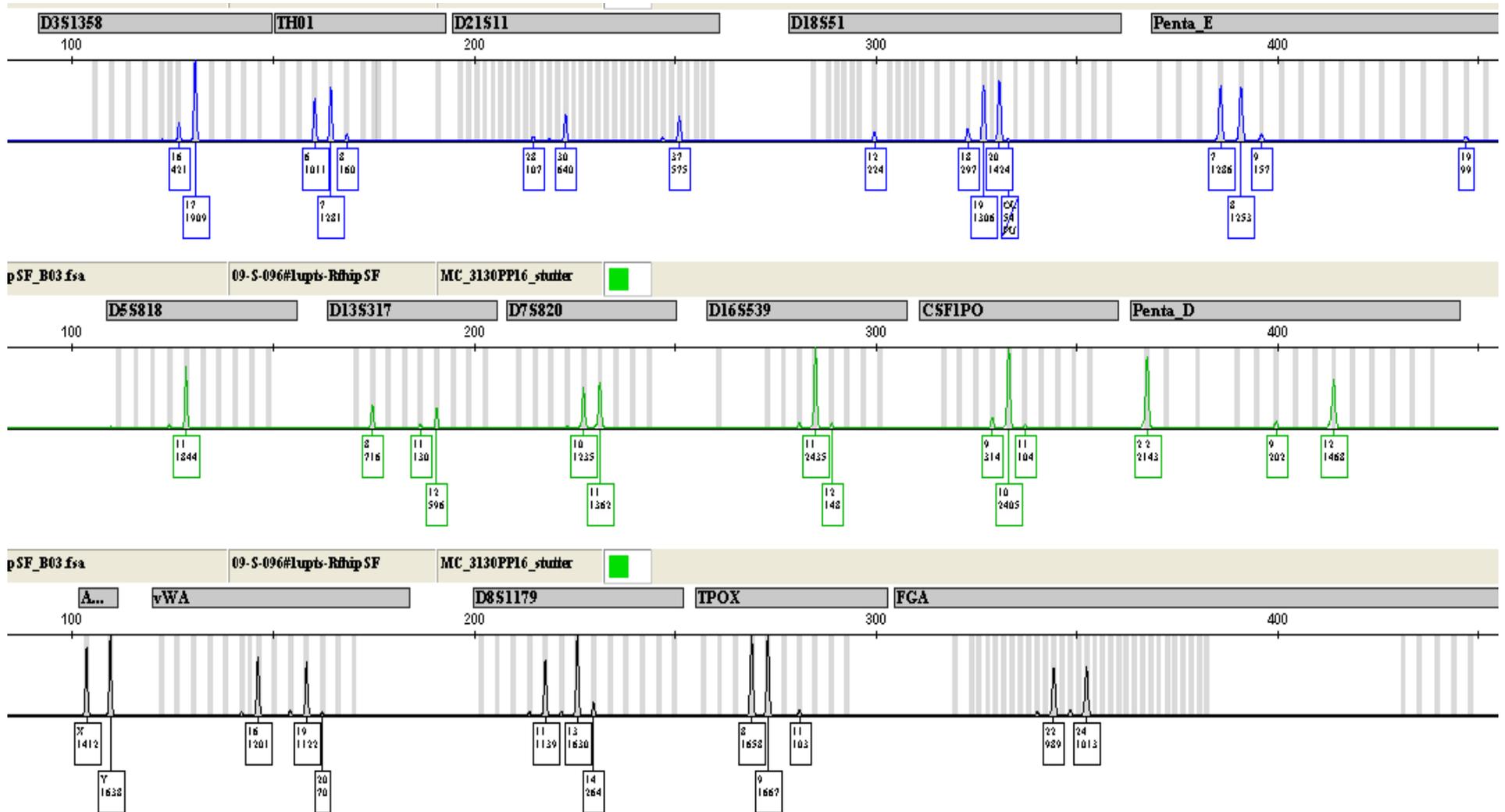
Blow up of D3, TH01, D21, D18 and Penta E
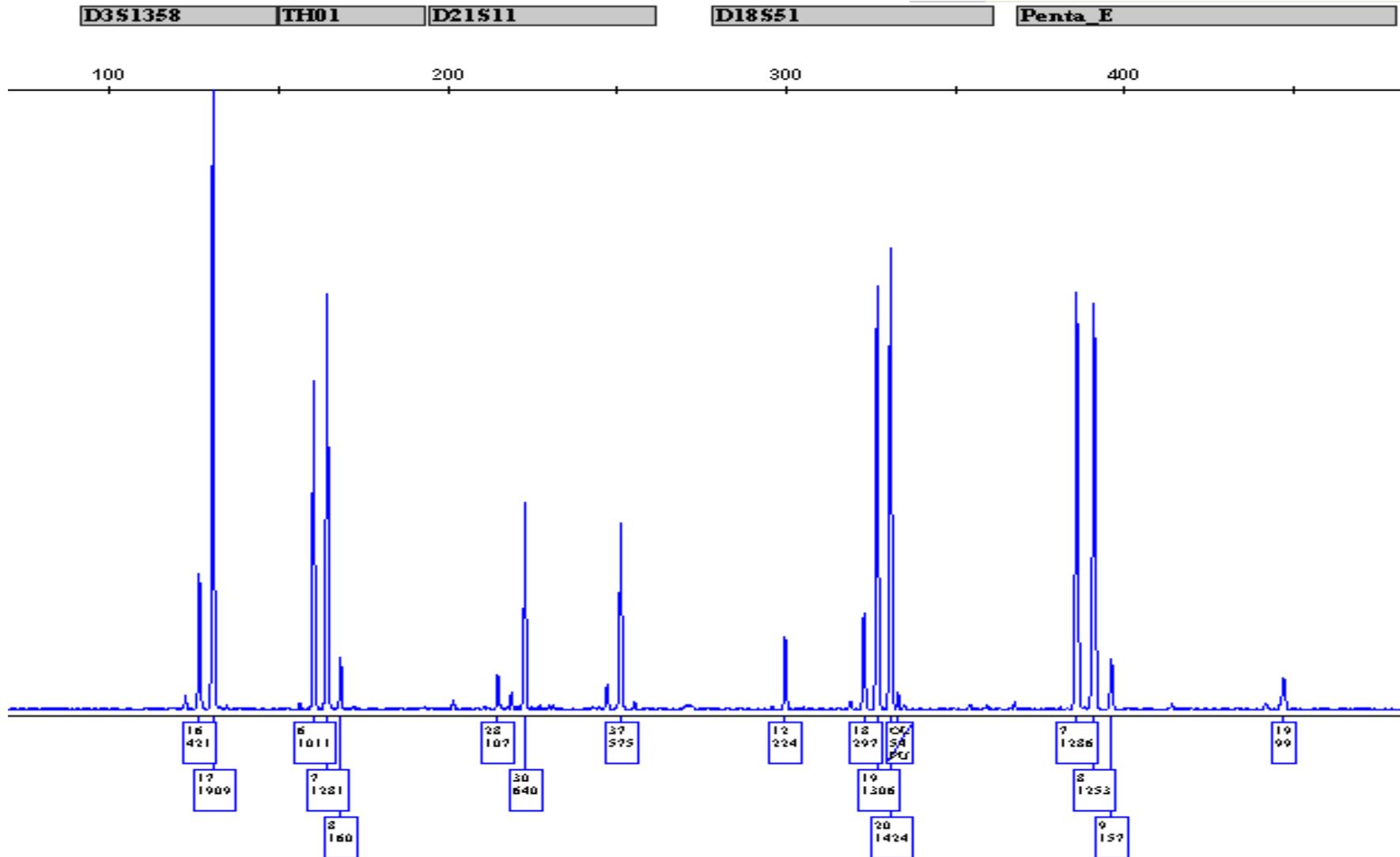
Blow up of D5, D13, D7, D16, CSF and Penta D
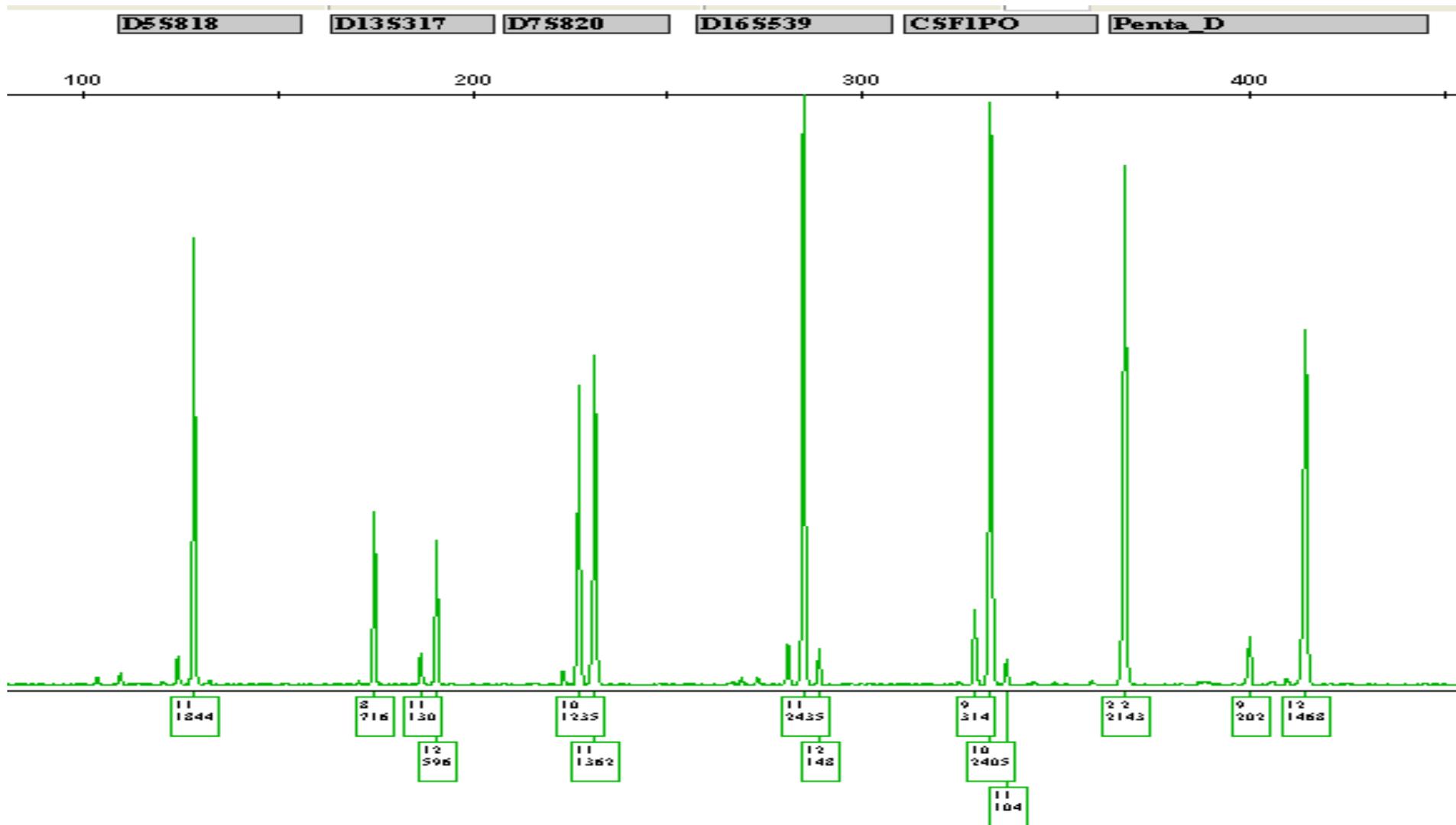
Blow up of Amelo, vWA, D8, TPOX and FGA
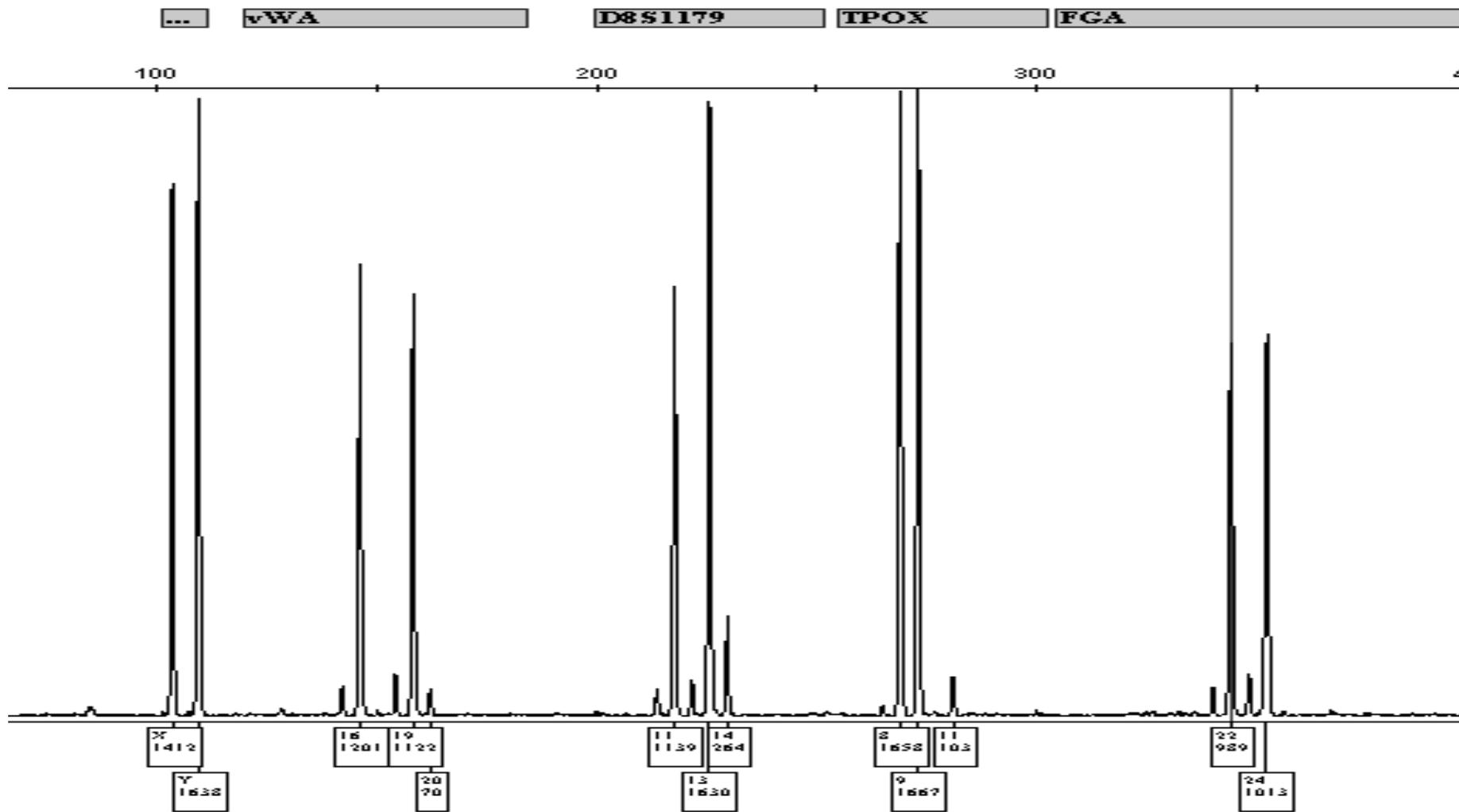
Initial Observations

- Mixture of at least two individuals based on maximum allele count

- The item (complainant's underpants) is considered an "intimate" item of evidence.

- The minor DNA types are not consistent with carry over from the complainant.

- Begin mixture deconvolution using our mixture worksheet.

Determine the mass ratio

- Use the loci with 4 alleles

- $\dfrac{(1306 + 1424)}{(224 + 297)}$

MR = 5.2

D18S51
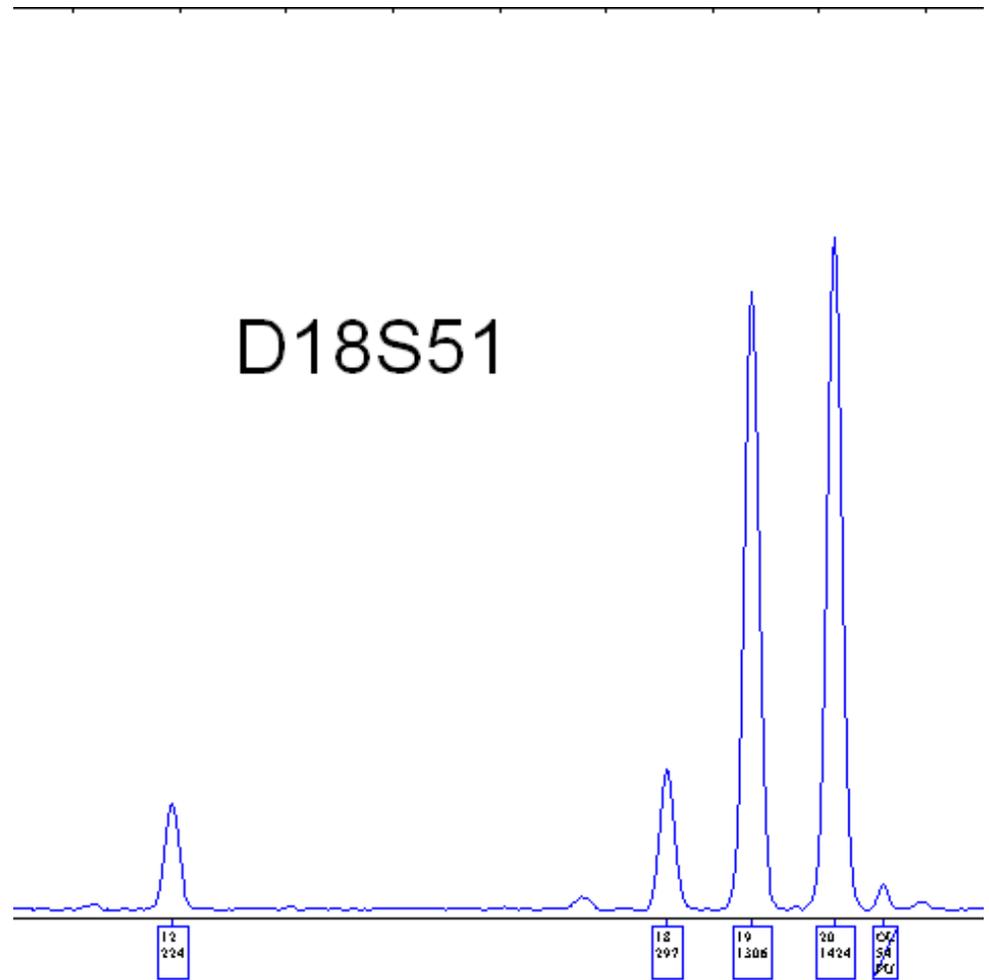
12 (224 rfu), 18 (297 rfu), 19 (1306 rfu), 20 (1424 rfu)

Determine the mass ratio

- Use the loci with 4 alleles

- $\dfrac{(1286 + 1253)}{(157 + 99)}$

MR = 9.9

Penta E
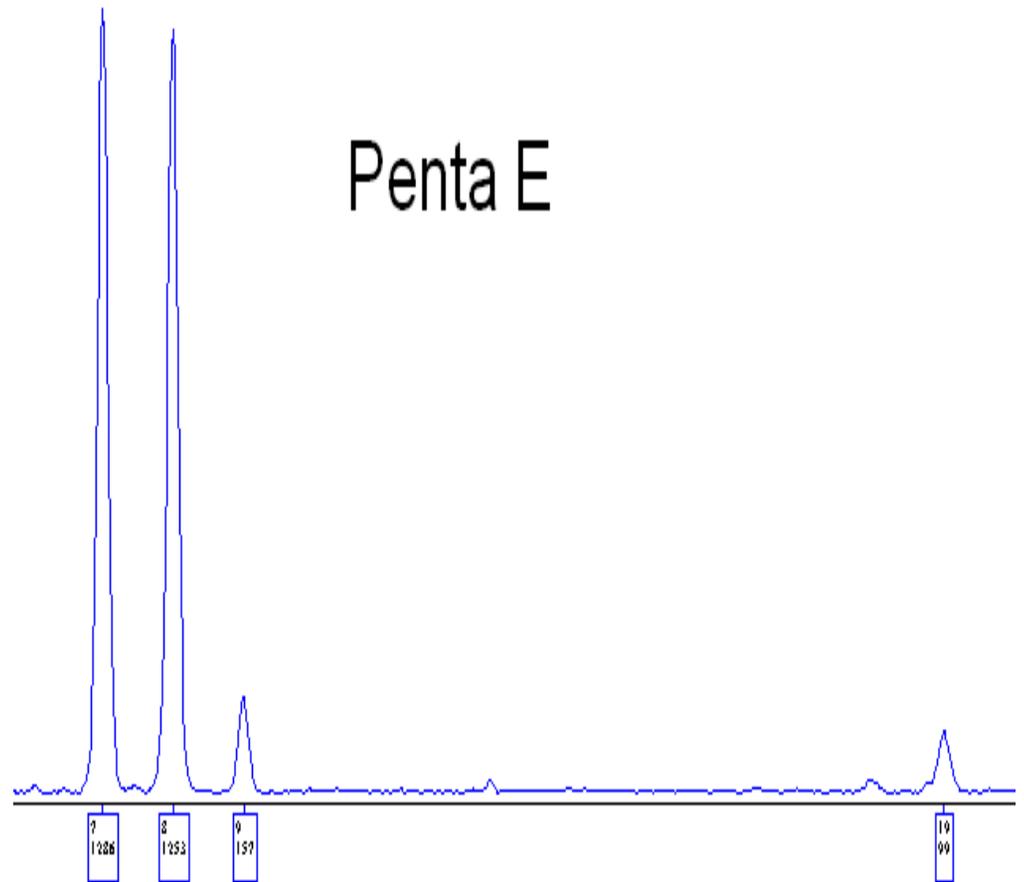
7 (1286 rfu), 8 (1253 rfu), 9 (157 rfu), 19 (99 rfu)

Mass Ratio

- Average the two loci to determine the overall mass ratio

- MR is ~ 7.5

- MR greater than 4:1 so the major profile qualifies for RMP statistic

- The major profile must also show 60% PHB in order to use that locus in the RMP statistic

Minor Contributor

- Minor alleles are then evaluated to see if a distinct contributor is present
- If a distinct contributor is present, then the minor profile qualifies for an RMP stat
- In this example, the minor alleles are low level and most are below our stochastic threshold of 230 rfu
- RMP stat is out but now the minor is evaluated to see if any loci qualify for a Combined Probability of Inclusion statistic

Loci suitable for CPI stat

- D3S1358 locus

- Only one minor allele detected at 421 rfu

- All alleles at locus above 230 rfu

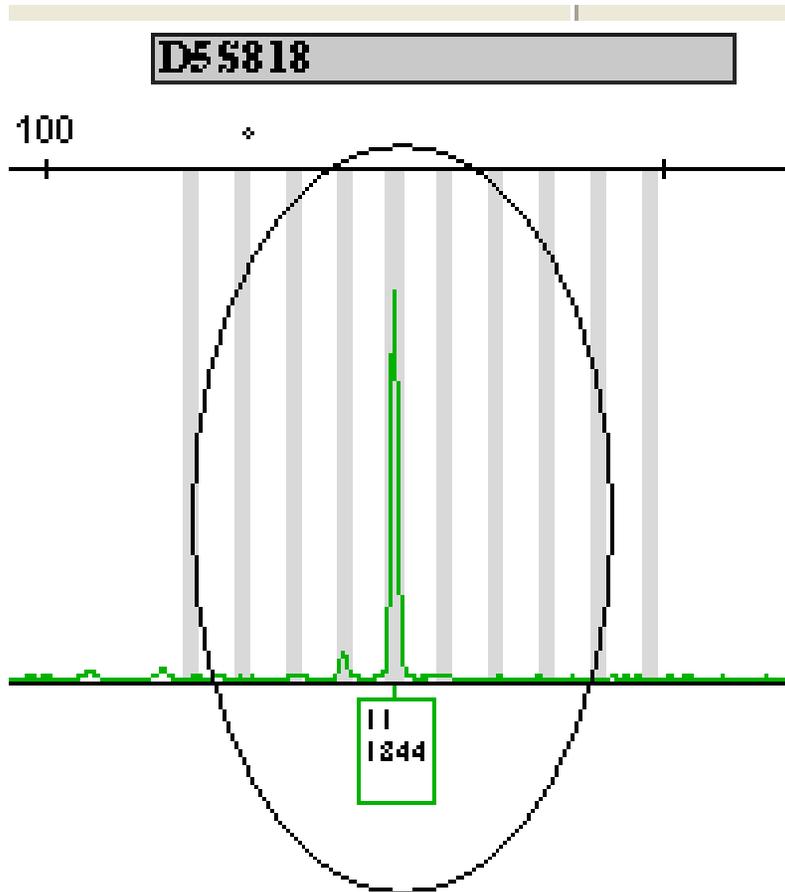- Locus qualifies for CPI stat
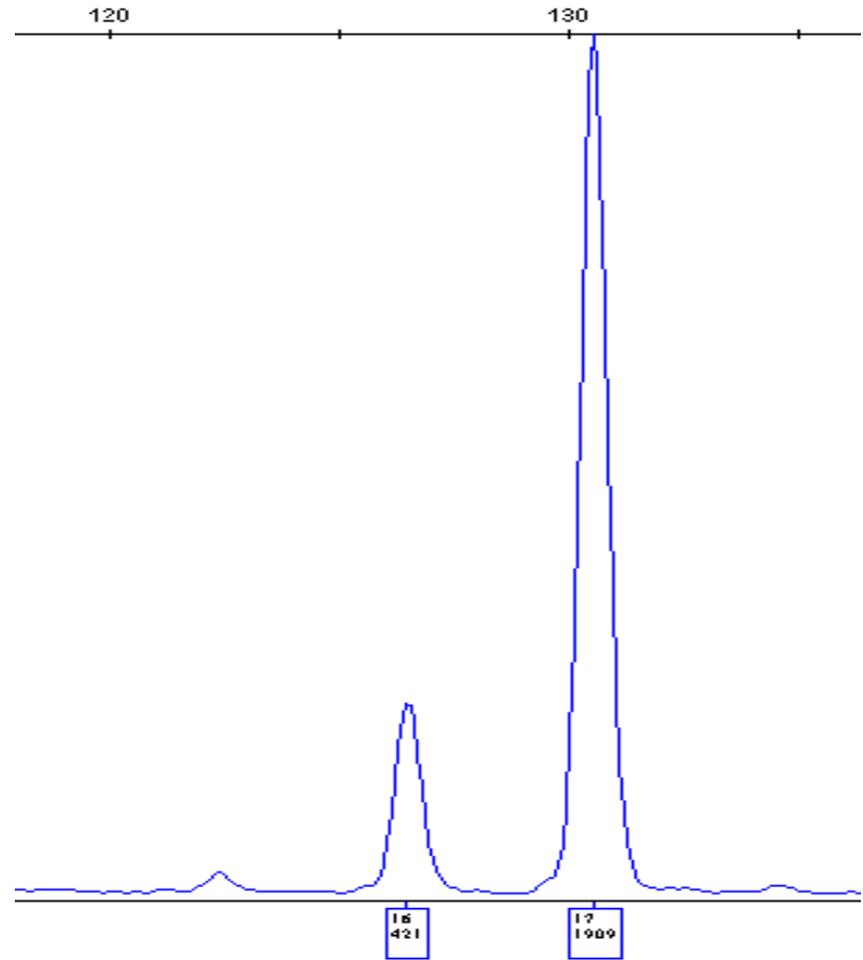
Loci suitable for CPI stat
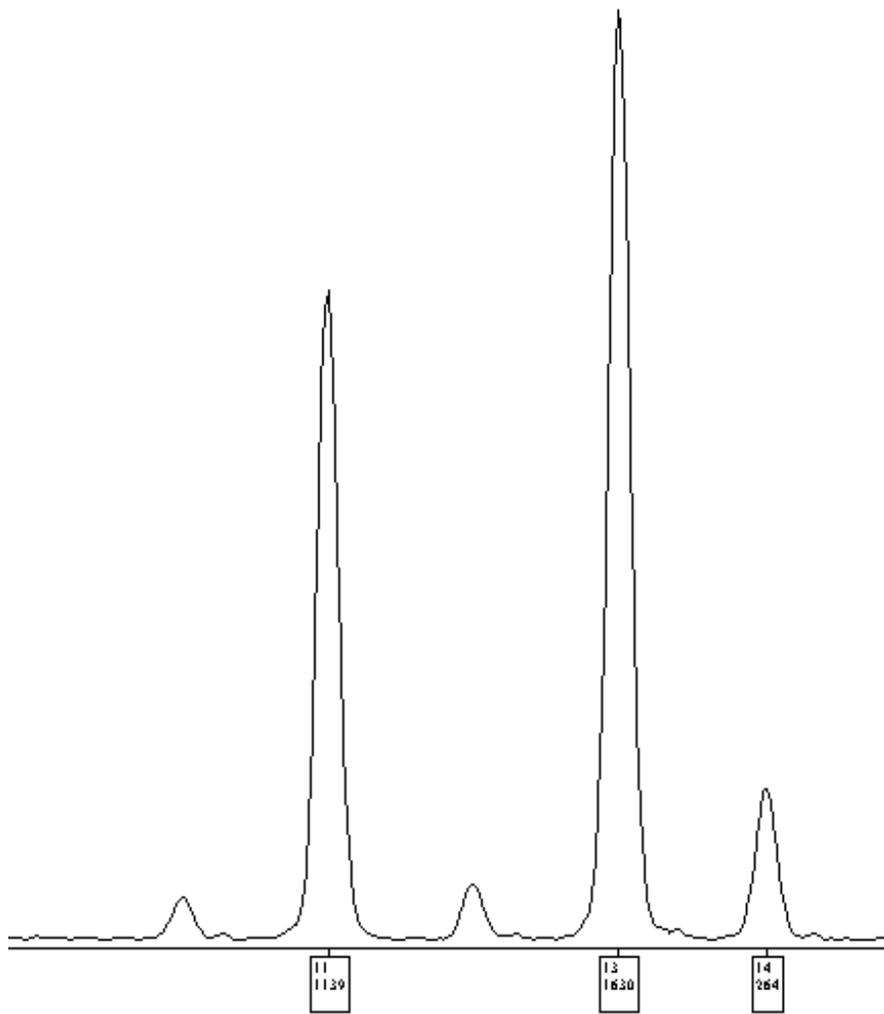
- D8S1179 locus

- Only one minor allele detected at 264 rfu

- All alleles at locus above 230 rfu

- Locus qualifies for CPI stat

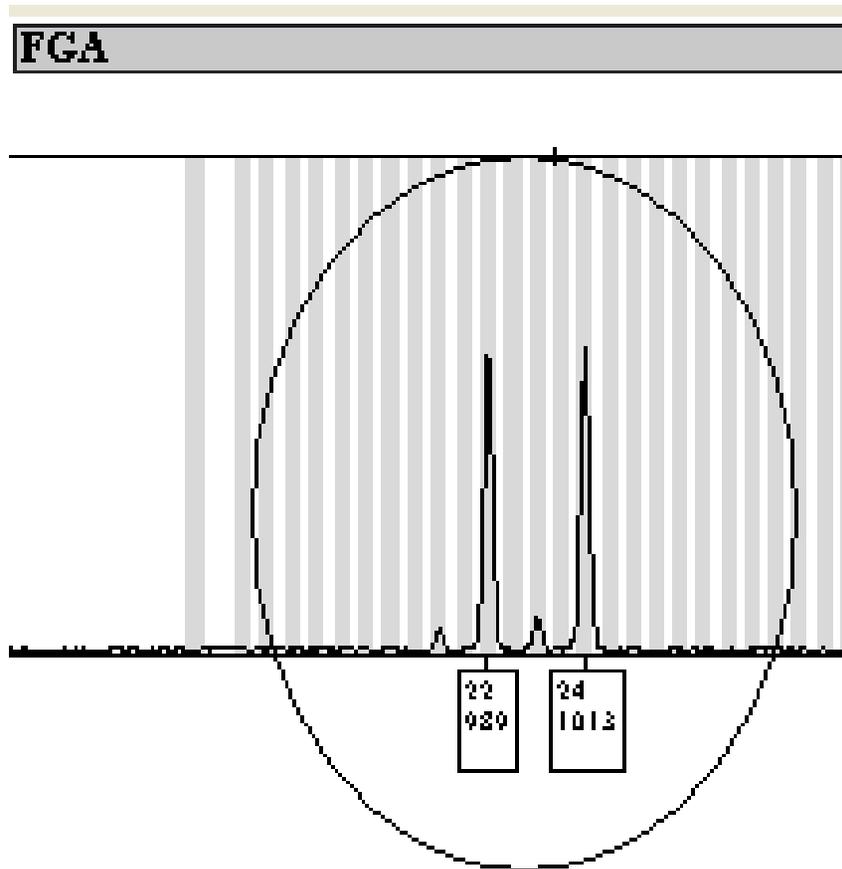# There are three additional loci that qualify for CPI stat
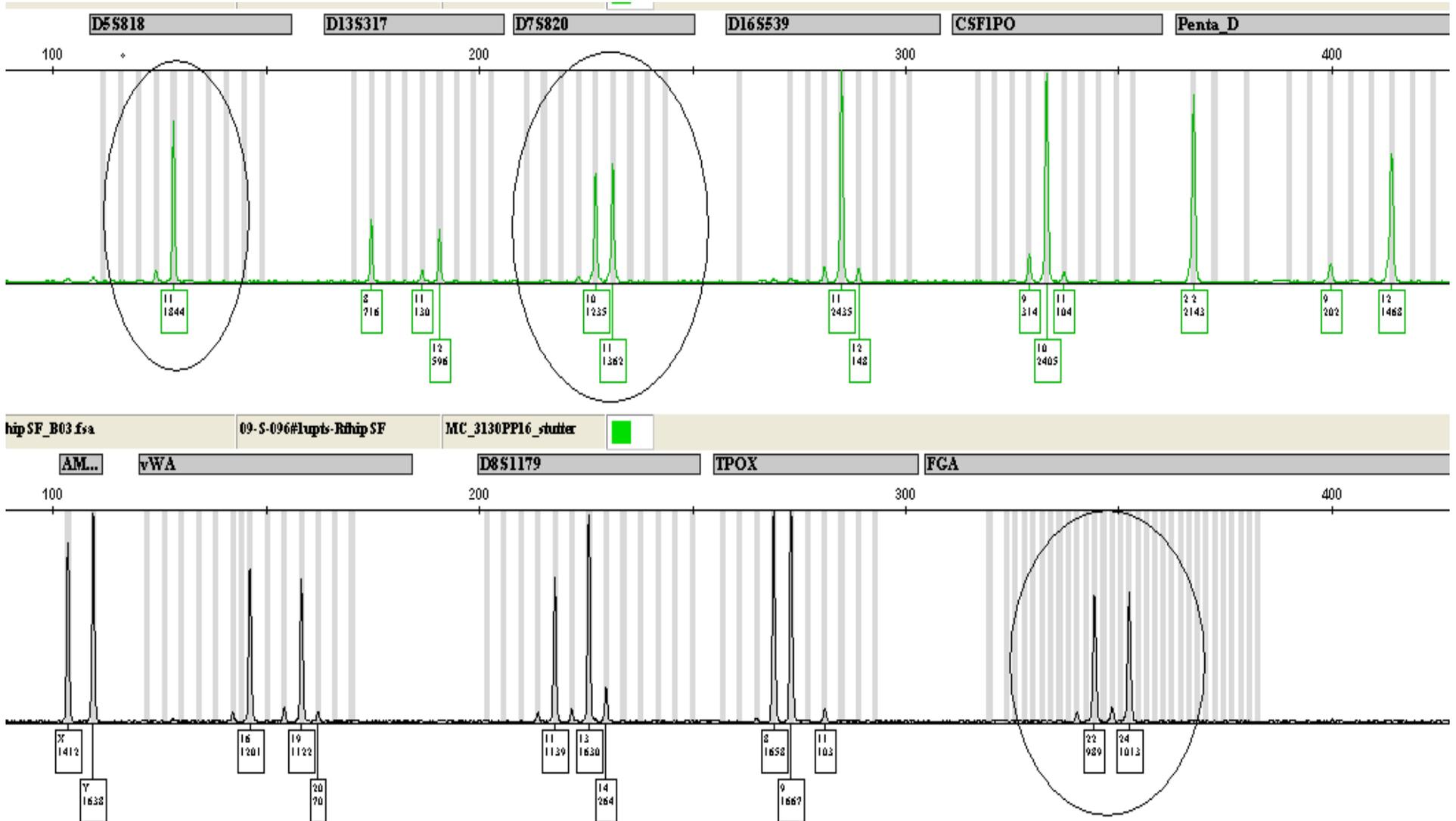
Use it for CPI?

- Only one allele detected at 1844 rfu

- Based on MR of ~7:5, the minor profile may not be completely represented

- Minor profile cannot be determined at this locus so no CPI stat

Use for CPI?

- Two alleles detected at 989 rfu and 1013 rfu

- Same issue as D5 so not used in the CPI stat

- Also the case for the D7 locus

Conclusions for the sperm fraction

- The major profile is different from Suspect #1 so it will be entered into CODIS. This profile was also detected on other samples.

- "A mixed DNA profile foreign to Complainant was obtained from the right hip area of the underpants. Suspect #1 is included as a contributor to the minor foreign DNA profile obtained from this sample."

- Major DNA profile addressed as being consistent with other samples.

Conclusions for the sperm fraction

- The probability of randomly selecting an unrelated individual who would be included as a contributor to this mixed DNA profile at the D3S1358 and D8S1179 loci is approximately:

  CAU   1 in 14 (Greater than 93.03% excluded)
  AFR   1 in 10 (Greater than 89.79% excluded)
  HIS   1 in 13 (Greater than 92.26% excluded)
  ASN   1 in 9   (Greater than 89.19% excluded)

- The remaining loci are consistent with Suspect #1 being a contributor to the minor foreign DNA profile obtained from the sperm fraction of the underpants. However, these loci did not meet the required interpretation criteria and were not used in the statistical calculation.

Table included in the report

SUMMARY OF POWERPLEX® 16 TYPING RESULTS

Item	Description	D3S1358	TH01	D21S11	D18S51	Penta E	D5S818	D13S317	D7S820	D16S539	CSF1PO	Penta D	Amelogenin	vWA	D8S1179	TPOX	FGA
1	Blood sample from Complainant	14,17	7,9	29,36	13,17.2	5	11	12,13	10	10,12	10,14	9,11	X	16,19	10,13	9,10	23,24
2	Buccal (cheek) sample from Suspect #1	16	7,8	24.3,38	12,18	9,19	11	11	11	11,12	9,11	22,9	XY	16,20	14	8,11	24
1	Sperm fraction from underpants	17(16)	6,7 (8*)	30,37 (28*)	19,20 (12*,18)	7,8 (9*,19*)	11	8,12(11*)	10,11	11(12*)	10 (9,11*)	22,12 (9*)	XY	16,19(20)	11,13 (14)	8,9 (11)	22,24
1	Non-sperm fraction from underpants	14,17	7,9	29,36	13,17.2	5	11	12,13	10	10,12	10,14	9,11	X	16,19	10,13	9,10	23,24

Key: Number and/or letters in parentheses ( ) represent alleles present in lesser intensity than other alleles not in parentheses.

* designates allele below stochastic threshold

Case Example #2

- Sexual Assault case
- One complainant and two suspects
- Boxer shorts submitted from Suspect #1
- No semen or blood was detected and a swab was taken from the front interior panel of the shorts
- Here is the DNA profile obtained from the front interior of the shorts:

Front interior of boxer shorts from Suspect
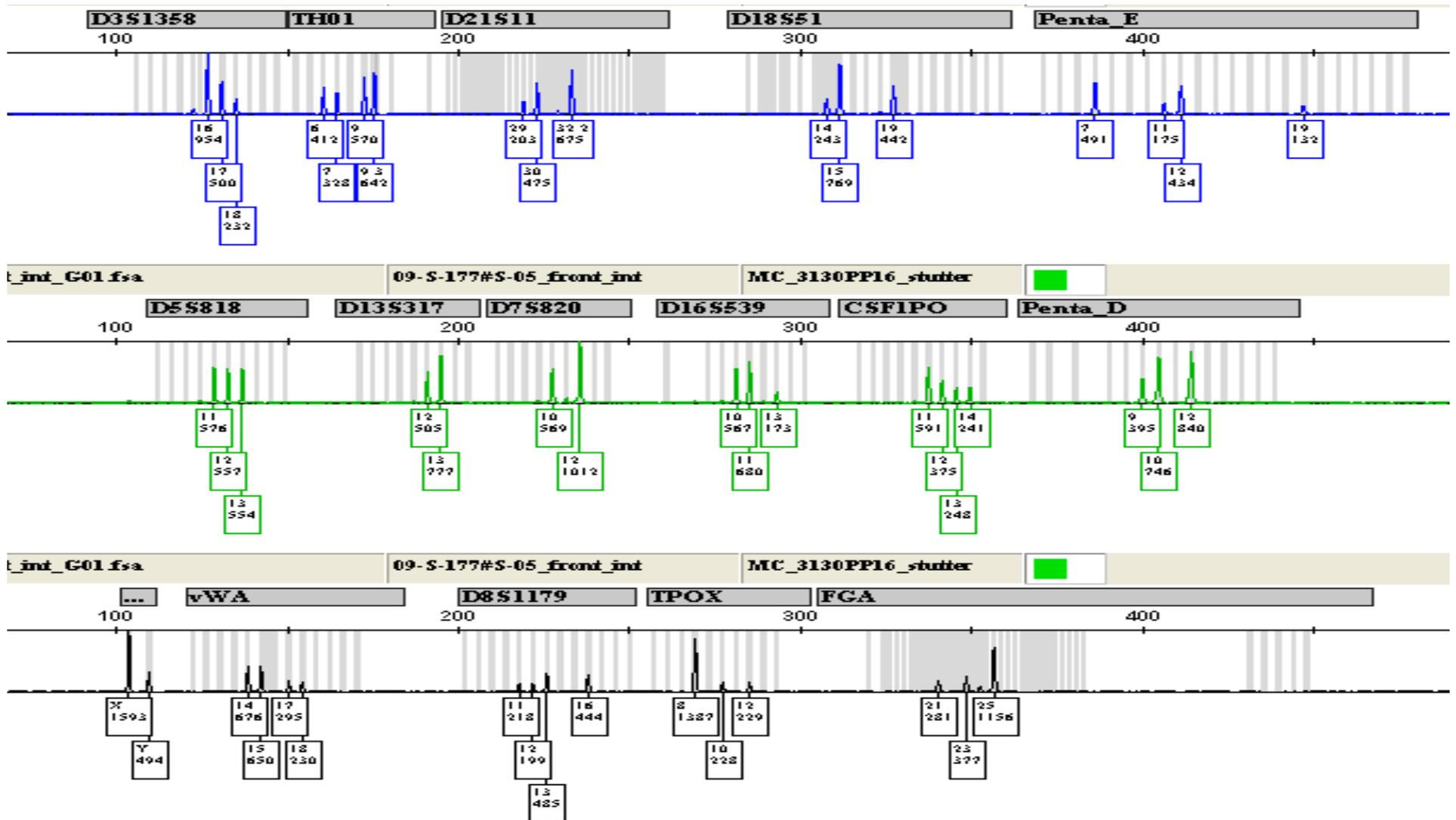
Assess overall mixture

- Evaluate entire mixture and determined its 2 contributors

- 6 (412), 7(328), 9(570), 9.3 (642)

- Divide the smallest (7) by the largest (9.3) and since below 60%PHB then determine MR (1.6)

TH01
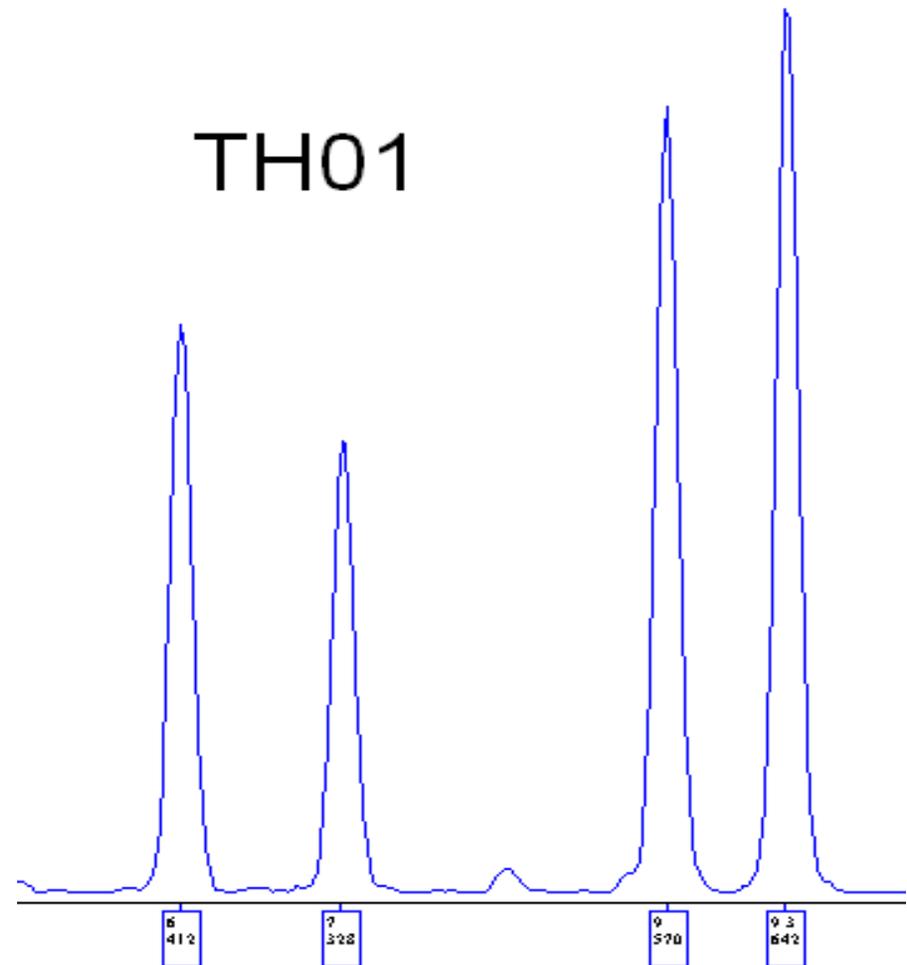
Mass Ratio

- Evaluate remaining 4 peak loci and overall mass ratio ~2.3

- MR < 4 so no major profile can be determined

- Boxer shorts considered an intimate item so a FVA can be used
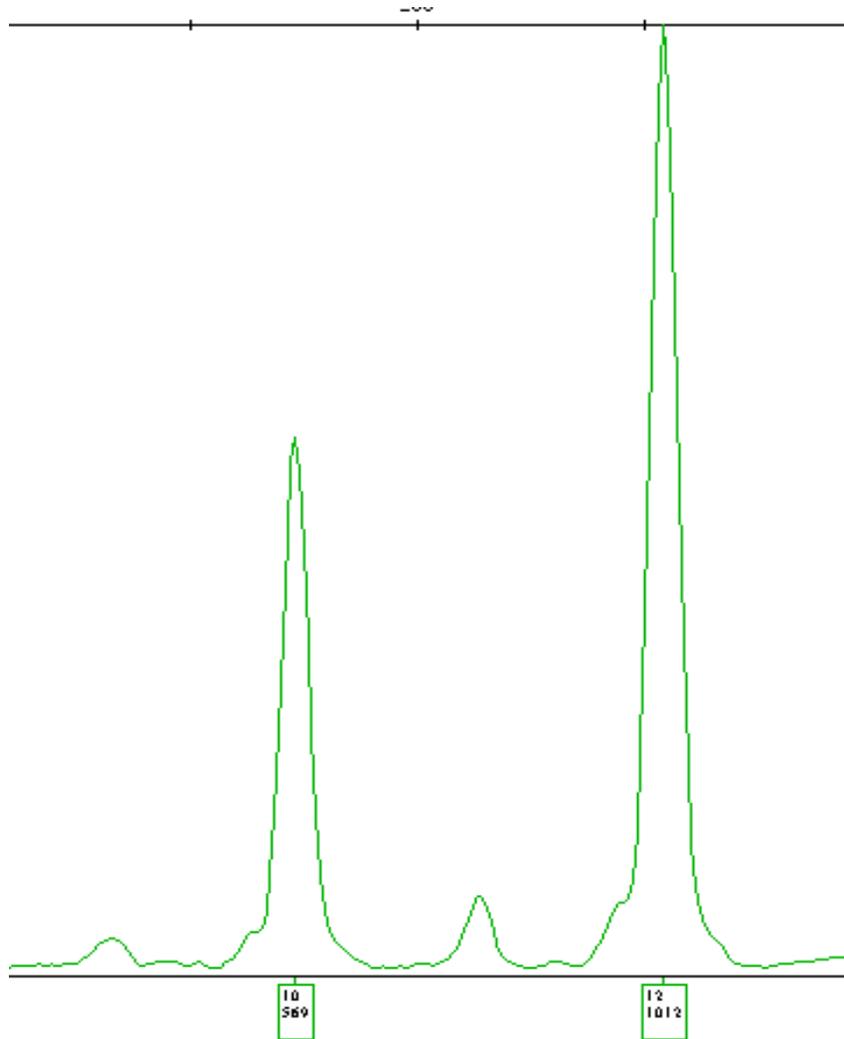
- Fill in the suspect's profile in our mixture worksheet

D3 locus

- Three alleles (16-954, 17-500, 18-232)
- Suspect = 16,18
- Use FVA:

  (16,17)/(16,18) = 2.2
- Fit Comparison [FC]:

  (500+232)/(954) = 77%

Deduced profile = 16,17
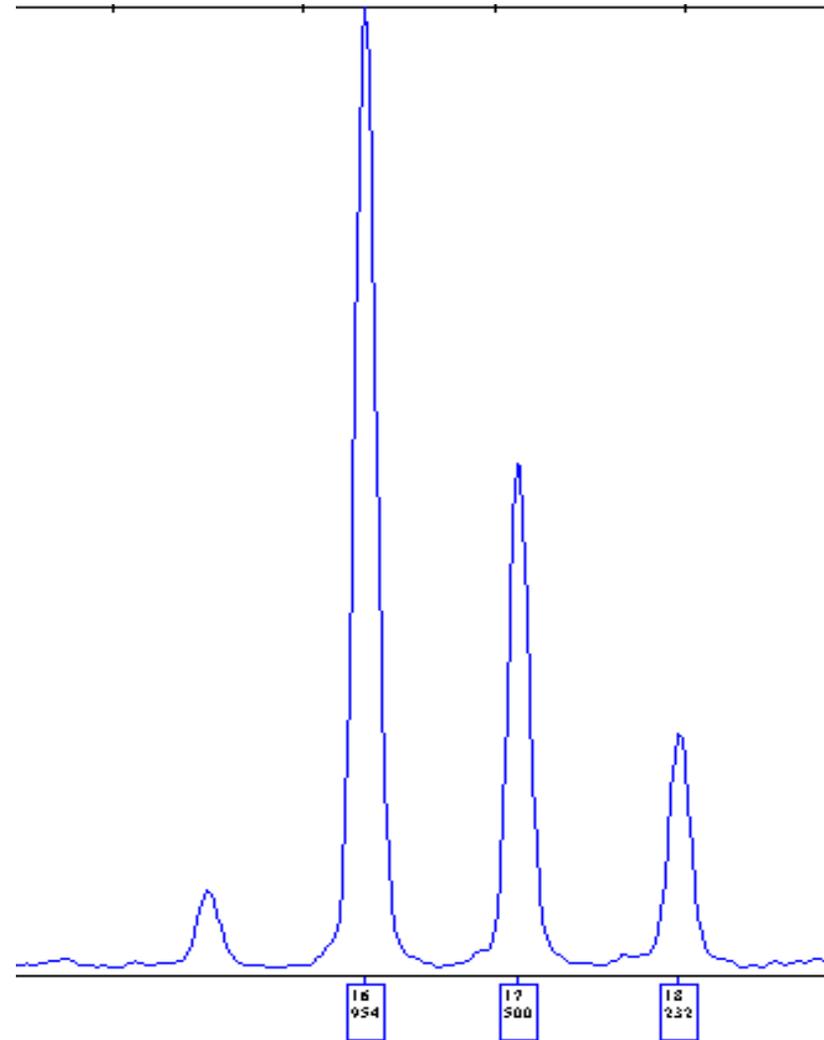
D7 locus

- 2 alleles (10-569 and 12-1012)
- Suspect = 12
- Use FVA:

  (10,12)/(12) = 2.6
- Deduced profile = 10,12 with a 100% PHB
- Suitable for RMP stat

Conclusions for Case #2

- "Assuming two contributors and Suspect #1 is one of the contributors, the Complainant is included as the other contributor to the mixed DNA profile obtained from the interior front panel of the boxer shorts from Suspect #1. Suspect #2 is excluded as a contributor to this mixed DNA profile."

Conclusions Case #2

- "The DNA profile from the Complainant matches the deduced DNA profile obtained from the interior front panel of the boxers from Suspect #1."

- "To a reasonable degree of scientific certainty, the Complainant is the source of the major DNA profile obtained from the interior front panel of the boxers from Suspect #1, with the exception of her identical twin."

Case Example #3

- Felony possession case
- Swab submitted from the gun
- Two suspects submitted for comparison
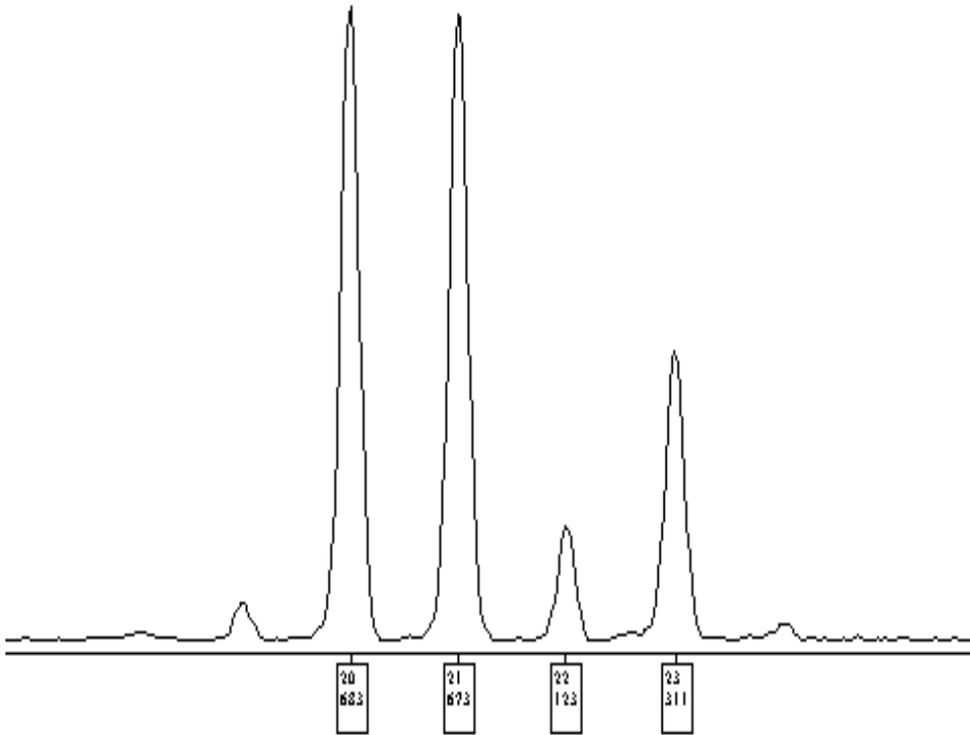
- Here is the profile from the gun:

Swab from gun
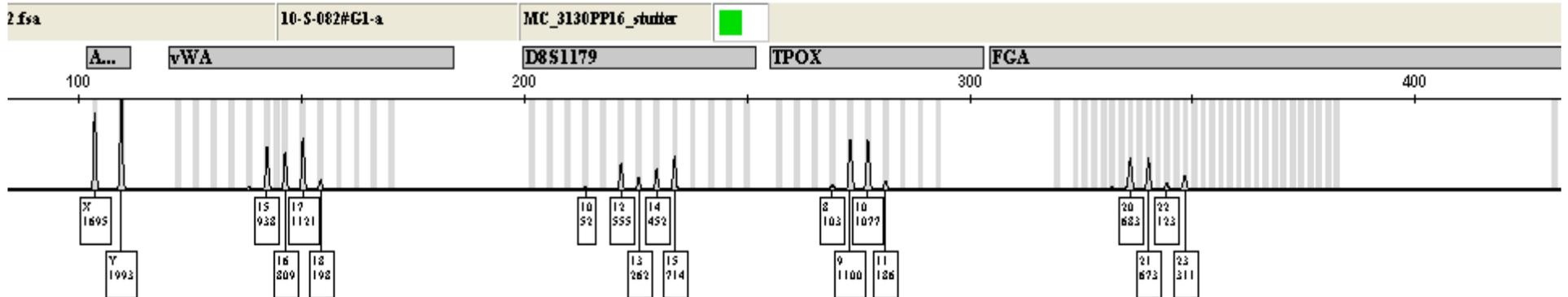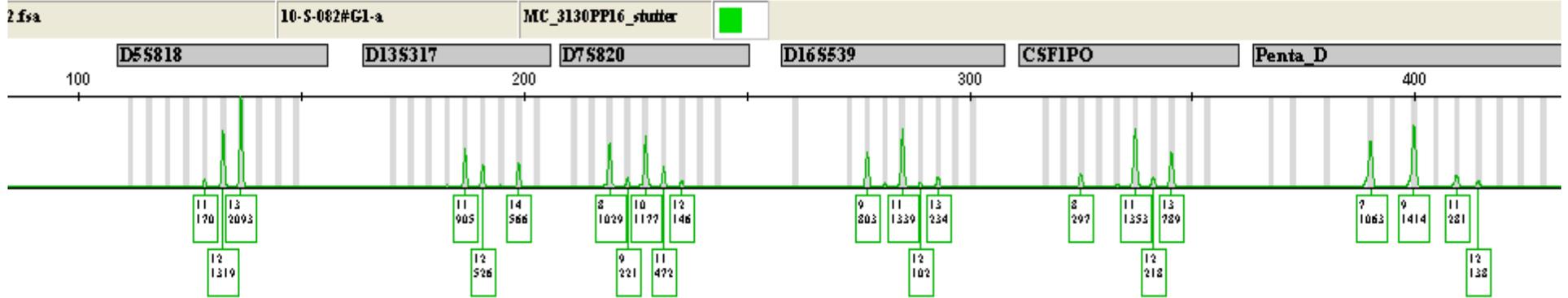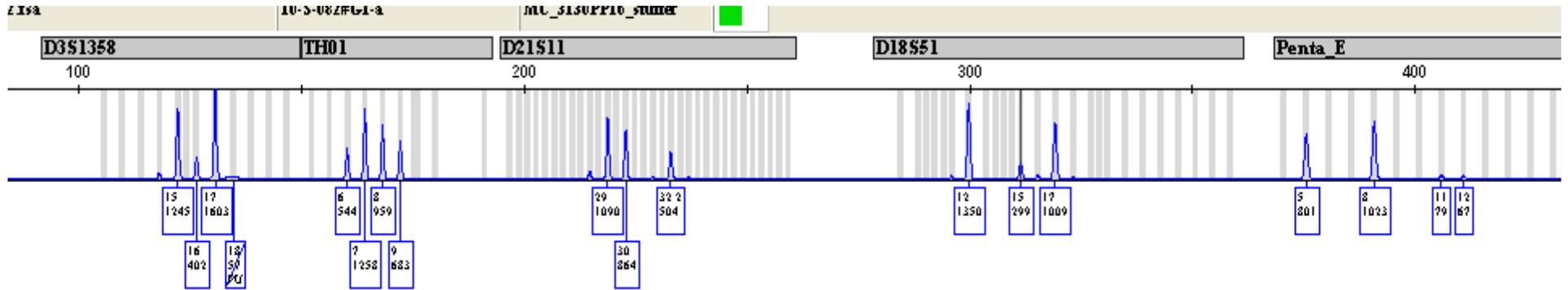
Assessment of mixture

- Evaluate number of alleles at each locus and 2 loci have 5

- Mixture of at least 3 contributors

- Evaluate the mixture to determine if there is a major contributor (mass ratio > 4:1)
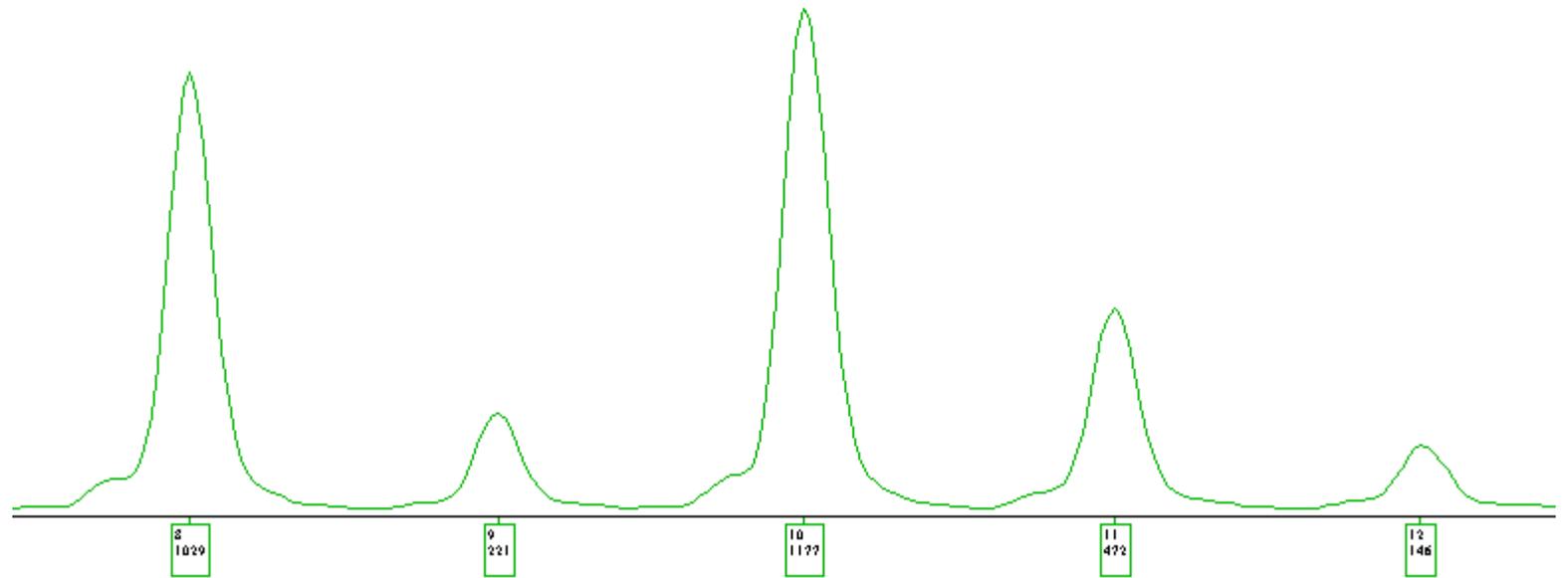
Blow up of FGA

- MR = <u>20,21</u>
  22,23
- MR = 3.1
- Examine overall mixture and not suitable to pull out a major

20 (683 rfu), 21 (673 rfu), 22 (123 rfu), 23 (311 rfu)

Blow up of D7S820

8 (1029 rfu) 9 (221 rfu) 10 (1177 rfu) 11 (472 rfu) 12 (146 rfu)

Conclusions

- A mixed DNA profile of at least three individuals was obtained from the sample.

- One suspect was included and the other excluded.

- CPI/CPE statistic calculated for D3, TH01, D21, D18 and D13 (all alleles need to be above stochastic threshold of 230)

Improvements

- Performed a material modification to increase the volume of sample and therefore allowing an increase in DNA input to the amplification reactions

- Target amount increased to 2.5ng and may exceed this amount based on profile rfus

- Increase in target concentration evaluated for varying mixture ratios

Improvements

- An increase in # of loci used for statistics was noted when increasing the target concentration for lower mass ratios (4 and below)

- More information was obtained (alleles above analytical threshold) when increasing the target concentration for higher mass ratios if the minor profile is probative

Improvements

- We now have the ability to select profiles (evidence and known samples if a FVA is used) and directly upload the alleles and rfus into the mixture worksheet
- Calculations are still being done manually but the mixtures are no longer being handwritten into the worksheets

Complex Mixtures

- Difficult mixtures identified and were re-evaluated to ease interpretation
- Mixture of at least 3 individuals
- Touch evidence
- Alleles hovering around the stochastic threshold and numerous peaks below the analytical threshold
- Mixture not suitable for statistics
- So now what???

Complex Mixtures

- Mixtures being qualified as suitable for elimination purposes
- After a year of launching with our new interpretation guidelines, these mixtures were identified as the area of most difficulty to evaluate
- New set of rules to be implemented to address these mixtures

Complex Mixtures

- Mixture of at least 3 contributors
- Touch evidence
- No loci suitable for statistics
- Numerous loci with alleles below stochastic and even alleles noted below the analytical threshold
- These mixtures may be reported as not suitable for comparison purposes.

Wrap Up

- Access the community for assistance in implementing these interpretation guidelines in your lab
  - Validation, Exercises, Manuals, Difficult areas
- Great benefits
  - Consistency between analysts and cases
  - Analysis time will decrease over time
- Absolutely worth the investment and I was a skeptic!

Acknowledgments

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