

# Implementation of YSTR Testing

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# Presentation Overview

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- BCA involvement with Y STRs
- Validations
- Case Acceptance Policy
- Case Stats
- Reporting Guidelines
- Court Experience
- Case Examples



# YSTR History at BCA

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- 1999 Evaluation of YPlex 6 (Y6)
- 2000-2003 Validation of Y5 & Y6
- 2003 On-line with Y5 & Y6
- 2003 Evaluation of PPY
- 2004 Evaluation-Validation of Yfiler
- 2005 On-line with Yfiler



## Why Y....

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Amylase, a constituent of saliva, was indicated on the vaginal swabs (Item 1B), said to be from Jane Doe.

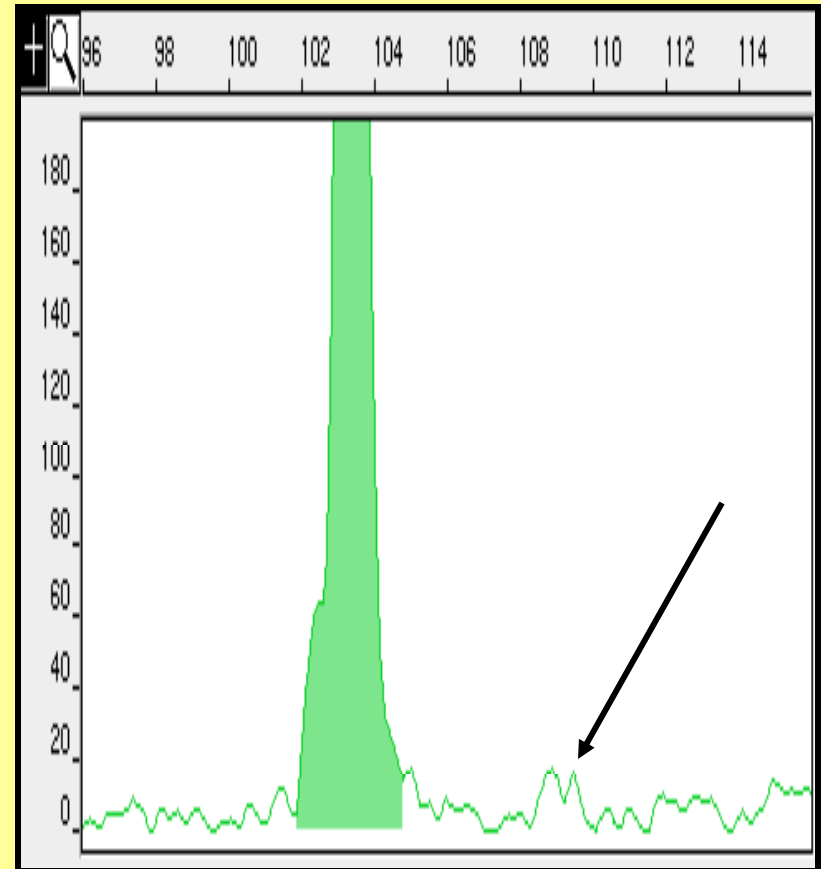
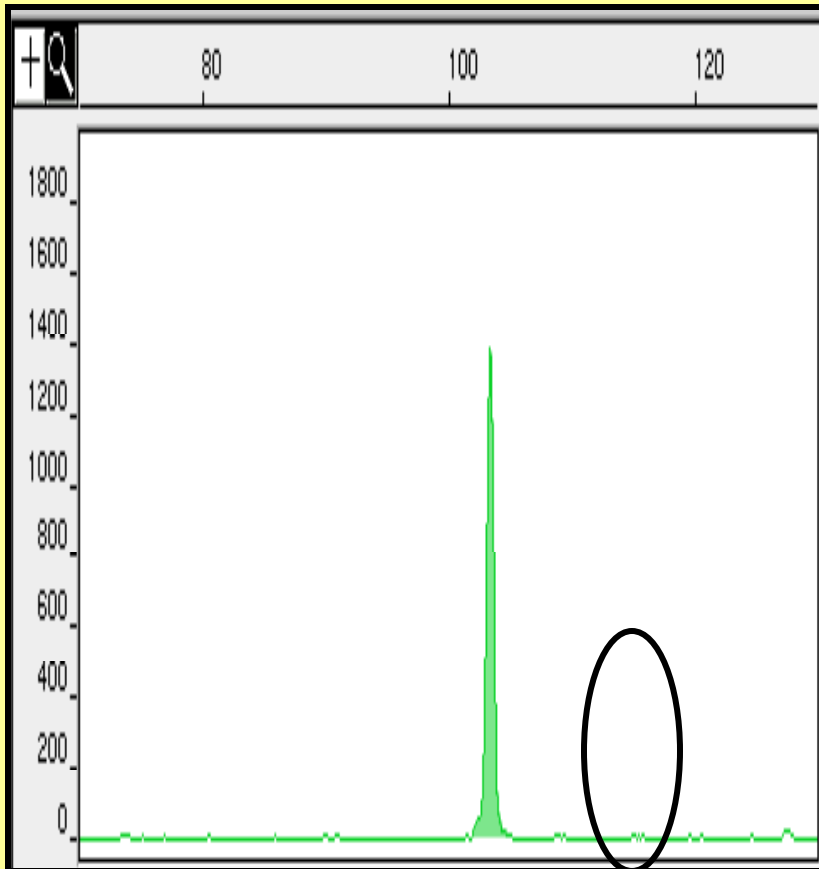


## Why Y.....

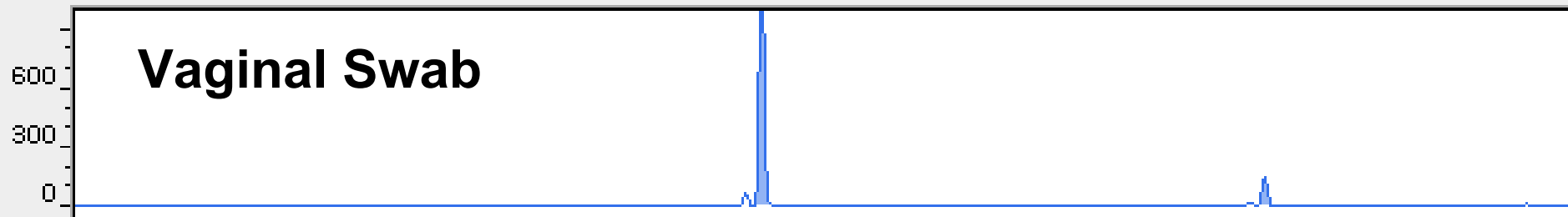
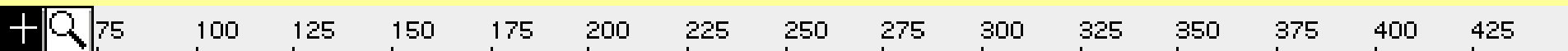
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No DNA types unlike those from Jane Doe were obtained from Item 1B. Therefore, no statement can be made regarding a possible source of the amylase indicated on this item.

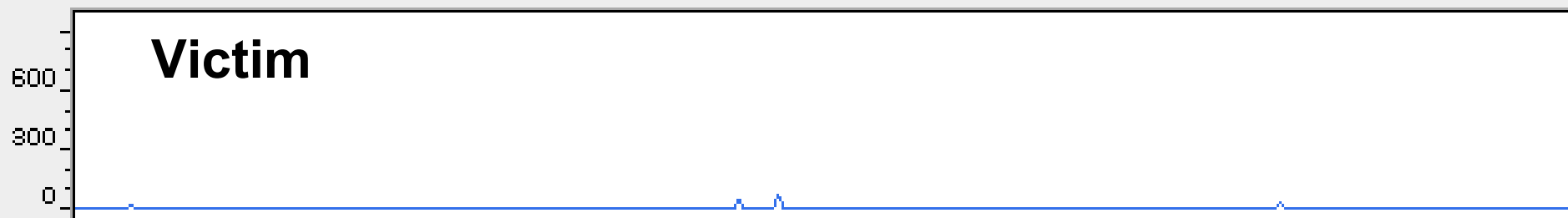
# Amelogenin Results



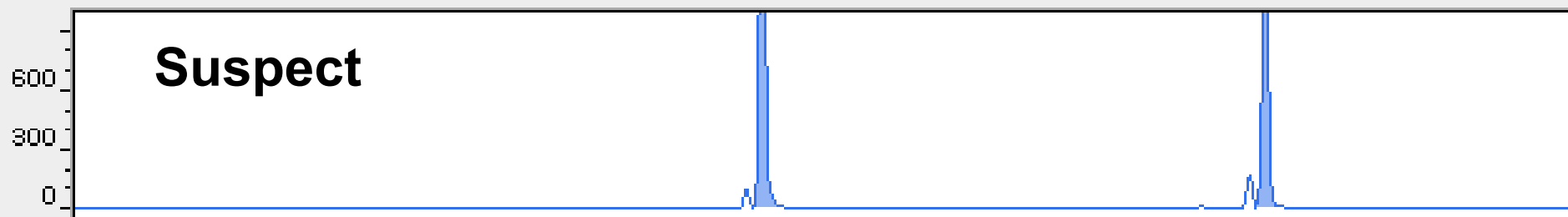
# Y5 Results – Case #1



15B :



3B :

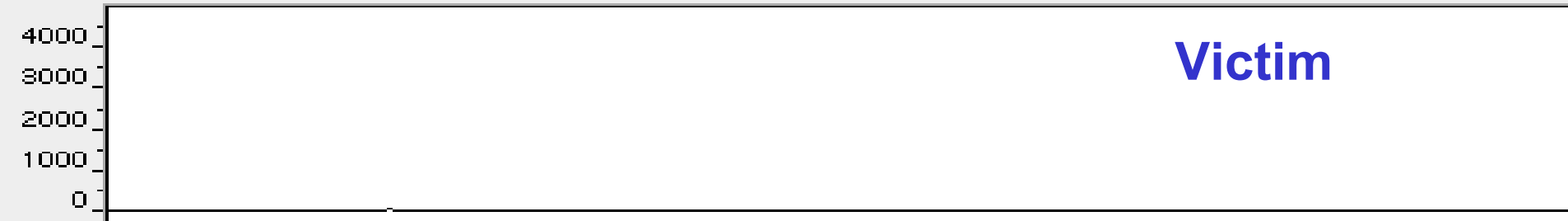


11B :

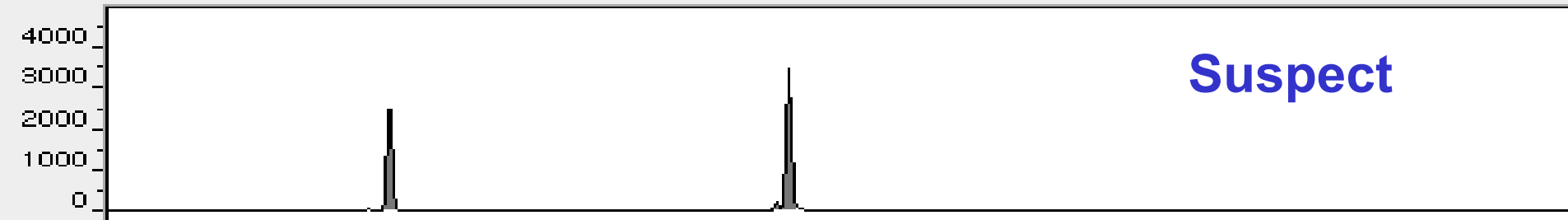
# Y5 Results – Case #1



15Y :



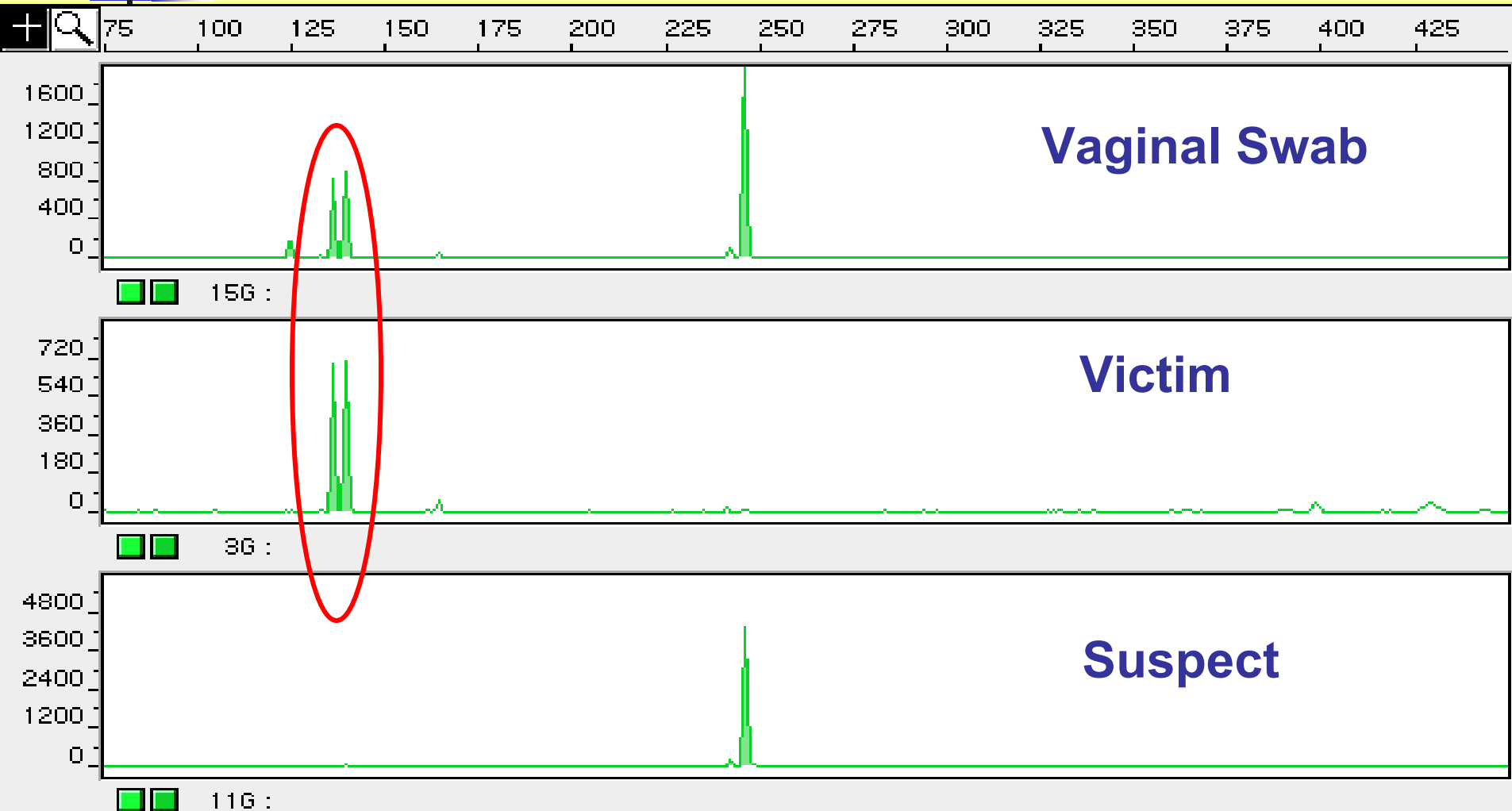
3Y :



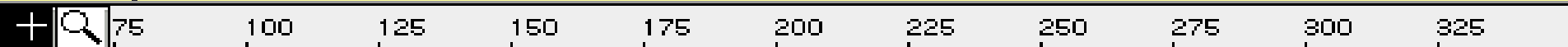
11Y :



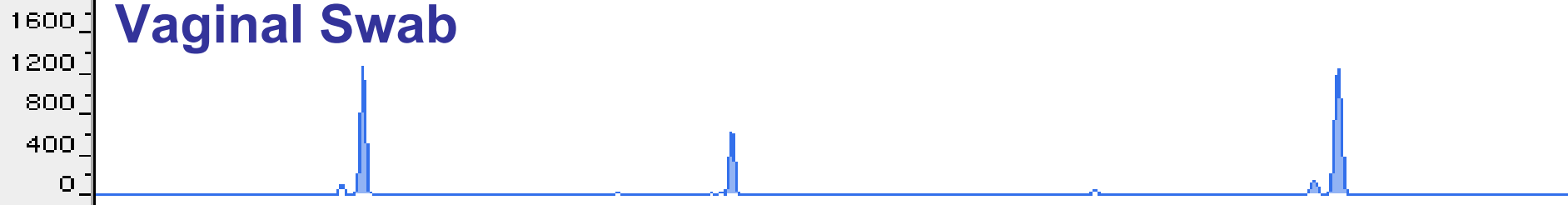
# Y5 Results – Case #1



# Y6 Results – Case #1



## Vaginal Swab



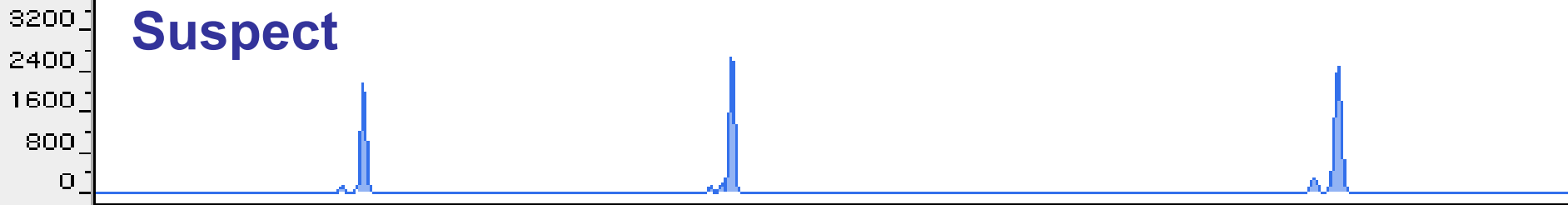
2B :

## Victim



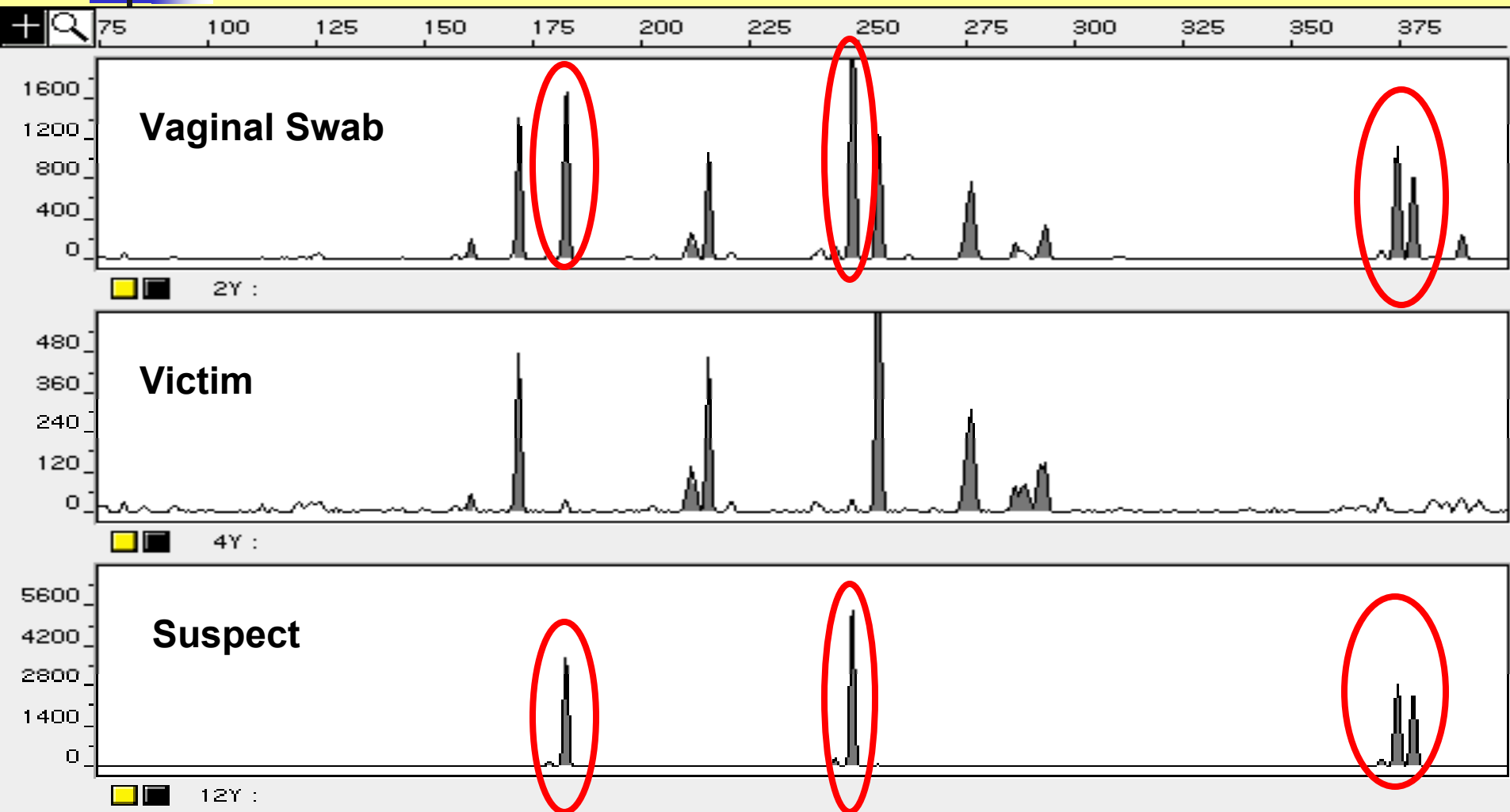
4B :

## Suspect

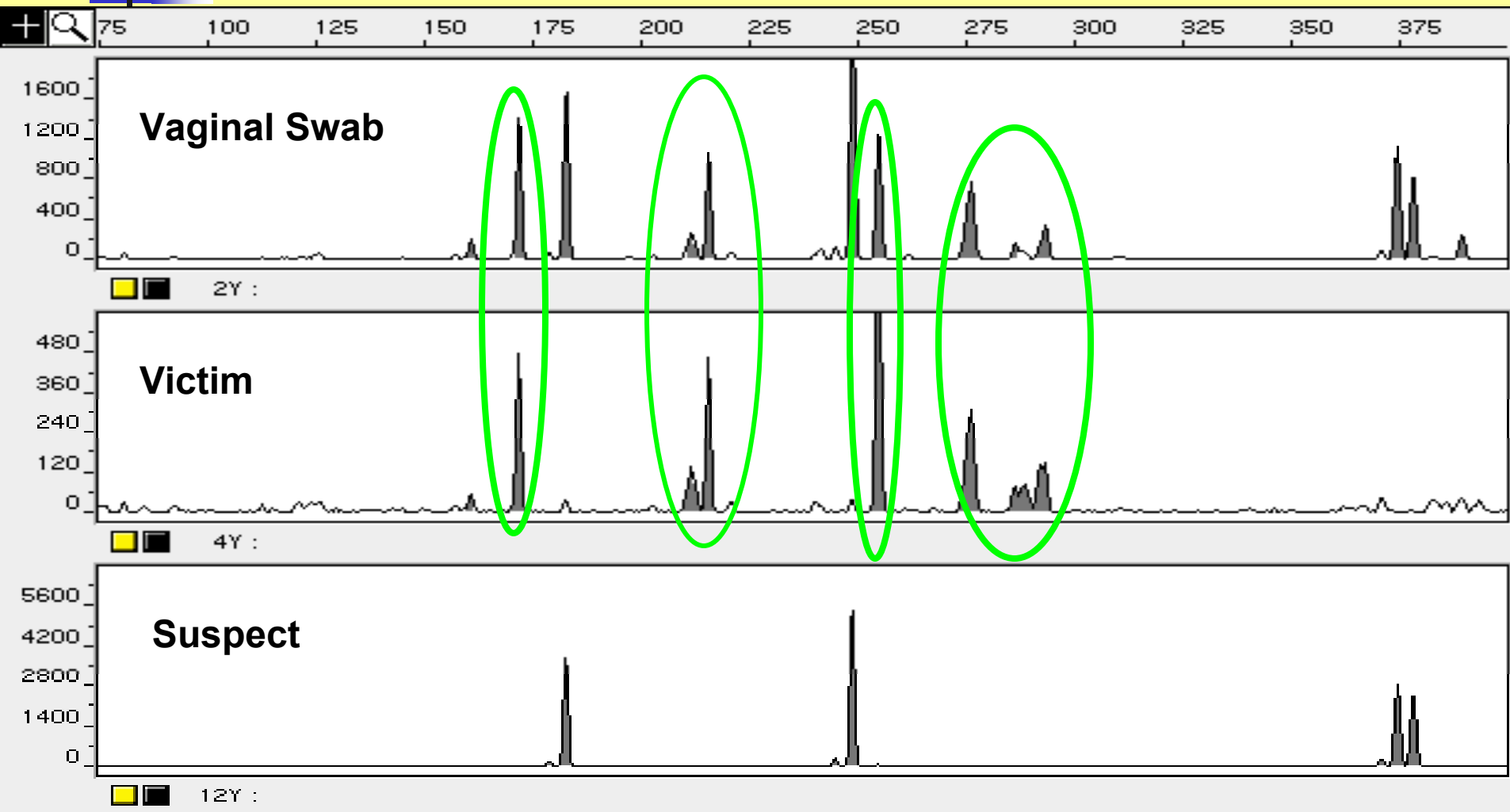


12B :

# Y6 Results – Case #1



# Y6 Results – Case #1





# Where we wanted to go.....

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- Single amplification
- Increased sensitivity
- Eliminate non-specific amplification
- More discrimination
- Male quantitation with Quantifiler Y



# Validations Completed at BCA

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- Concordance/Reproducibility
- Precision/Accuracy
- Stutter
- Sensitivity
- Female DNA
- Mixtures
  - Male:Male
  - Male:Female
- Adjudicated Cases

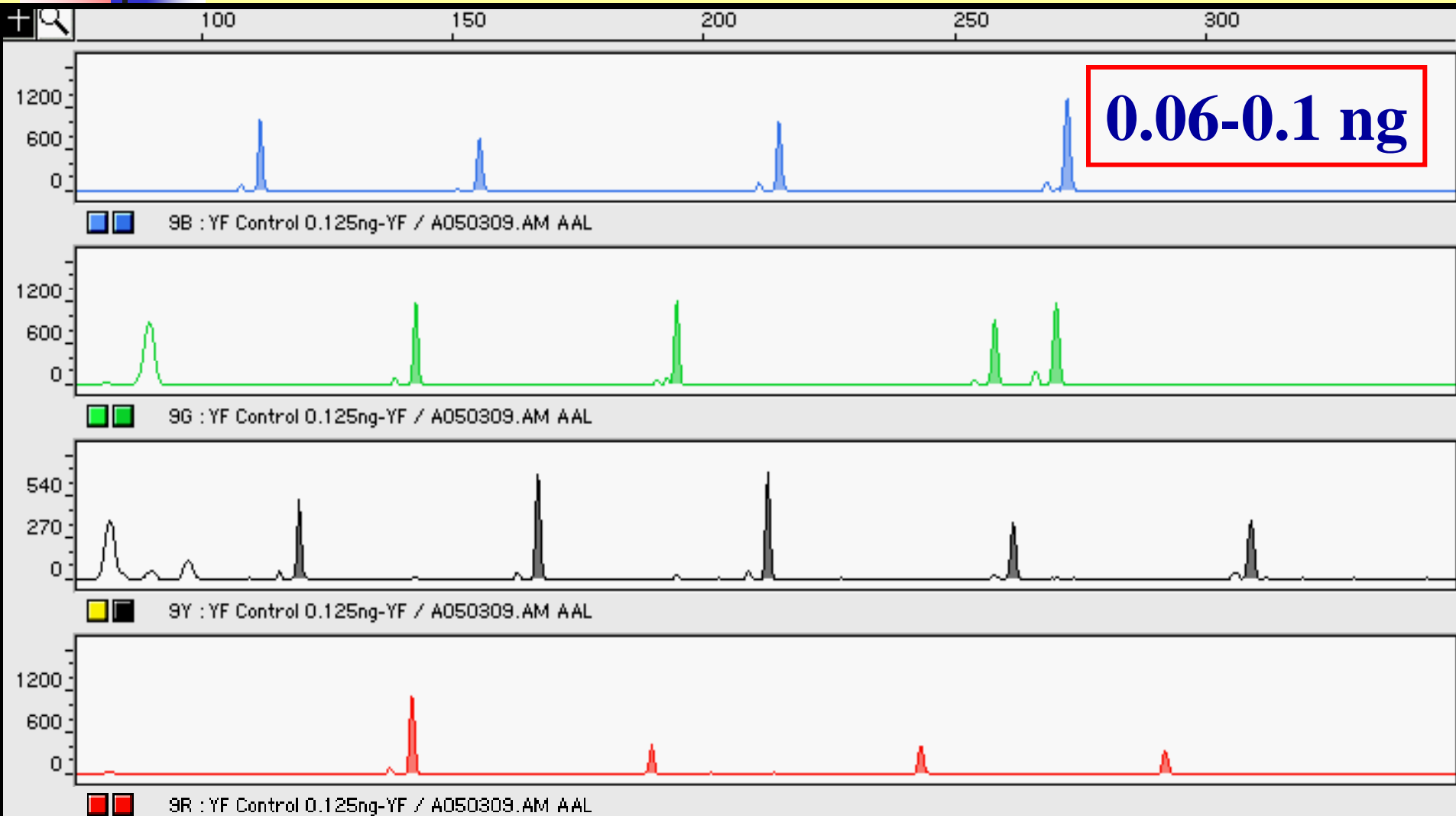


# Validations Completed at BCA

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# Sensitivity







# Sensitivity

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- Currently target 600-800pg male DNA for most samples.
- Exceptions
  - high amount of female DNA present
  - No result with Quantifiler Y



# Female DNA

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- Amplified 1000ng of 13 females
- Y5/Y6 showed variation among females
- Non-specific amplification was non-existent
- No need to amplify known female samples

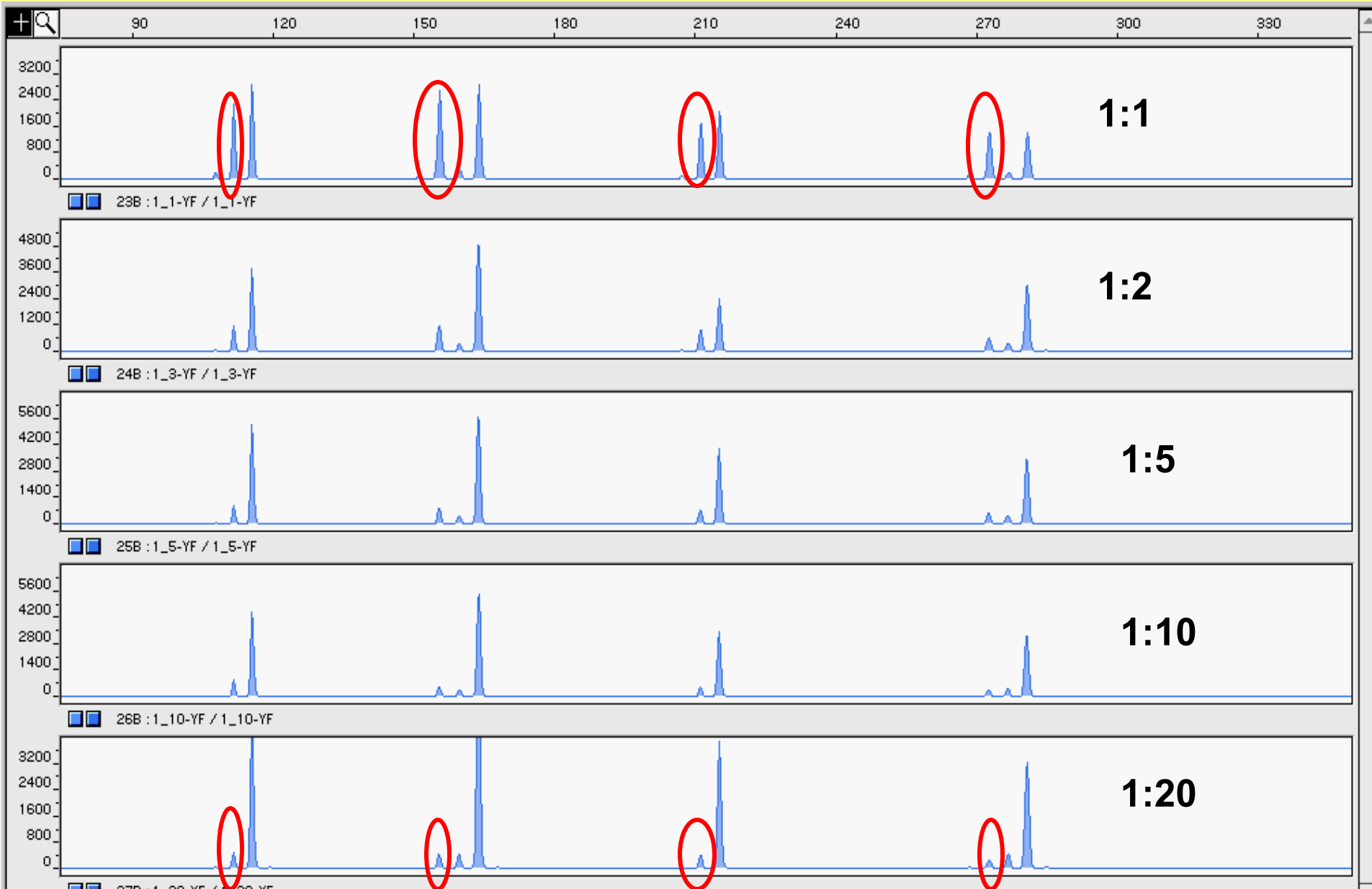


# Male/Male Mixtures

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- Tested a range from 1:1 to 1:20
- Minor contributor detected in the 1:20 mixtures
- Entire or near entire profile of minor contributor detected in the 1:10 mixtures

# Male/Male Mixtures



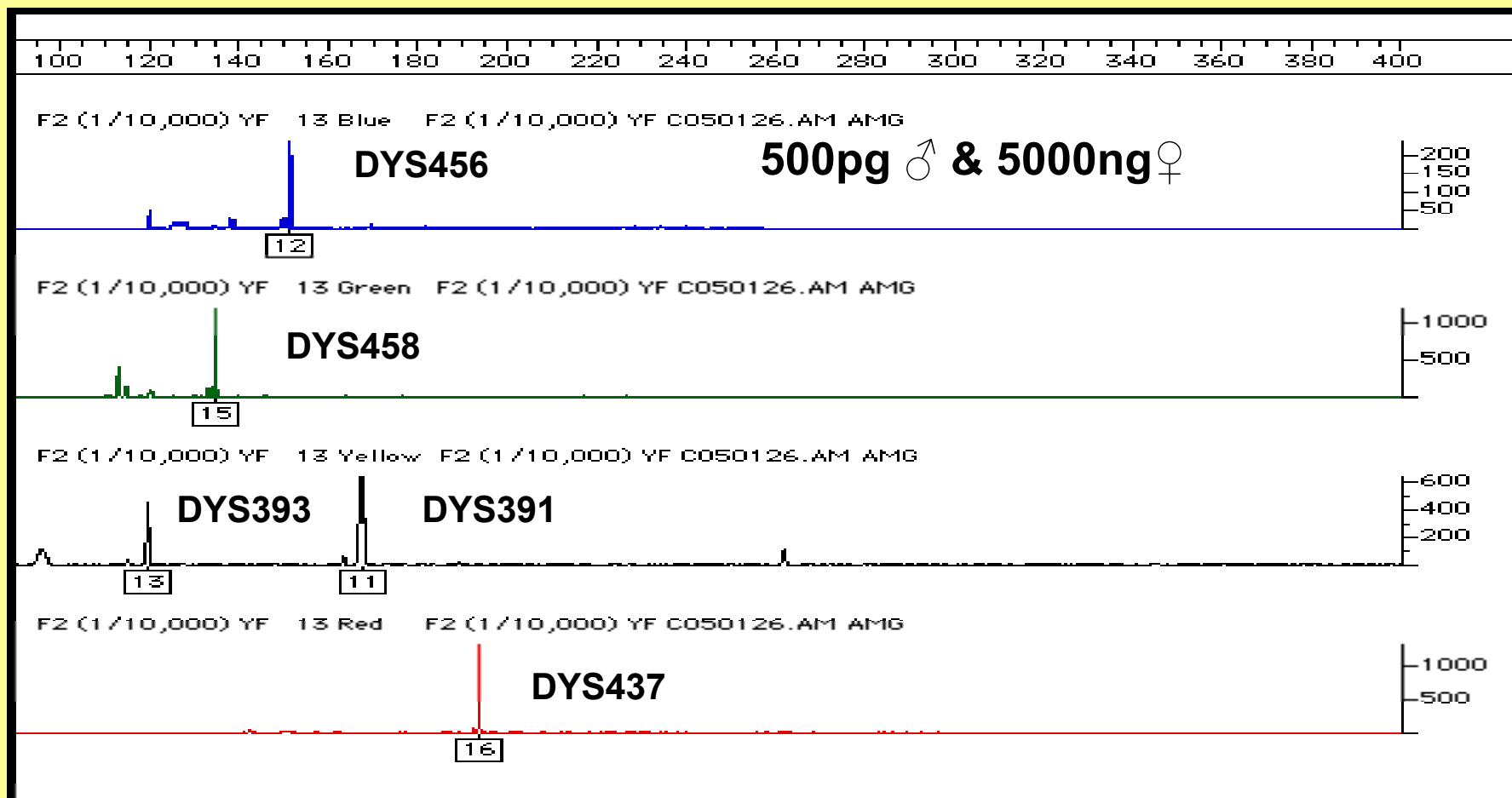


# Male/Female Mixtures I

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- 500pg male DNA mixed with increasing amounts of female DNA
- Up to 1:1000 – full profile
- 1:5000 mixture – one to three loci fell below the 150 RFU threshold
- 1:10,000 mixture – 12 of the loci were completely inhibited. The remaining 5 showed reduced peak heights.

# 1:10,000 mixture (Male:Female)



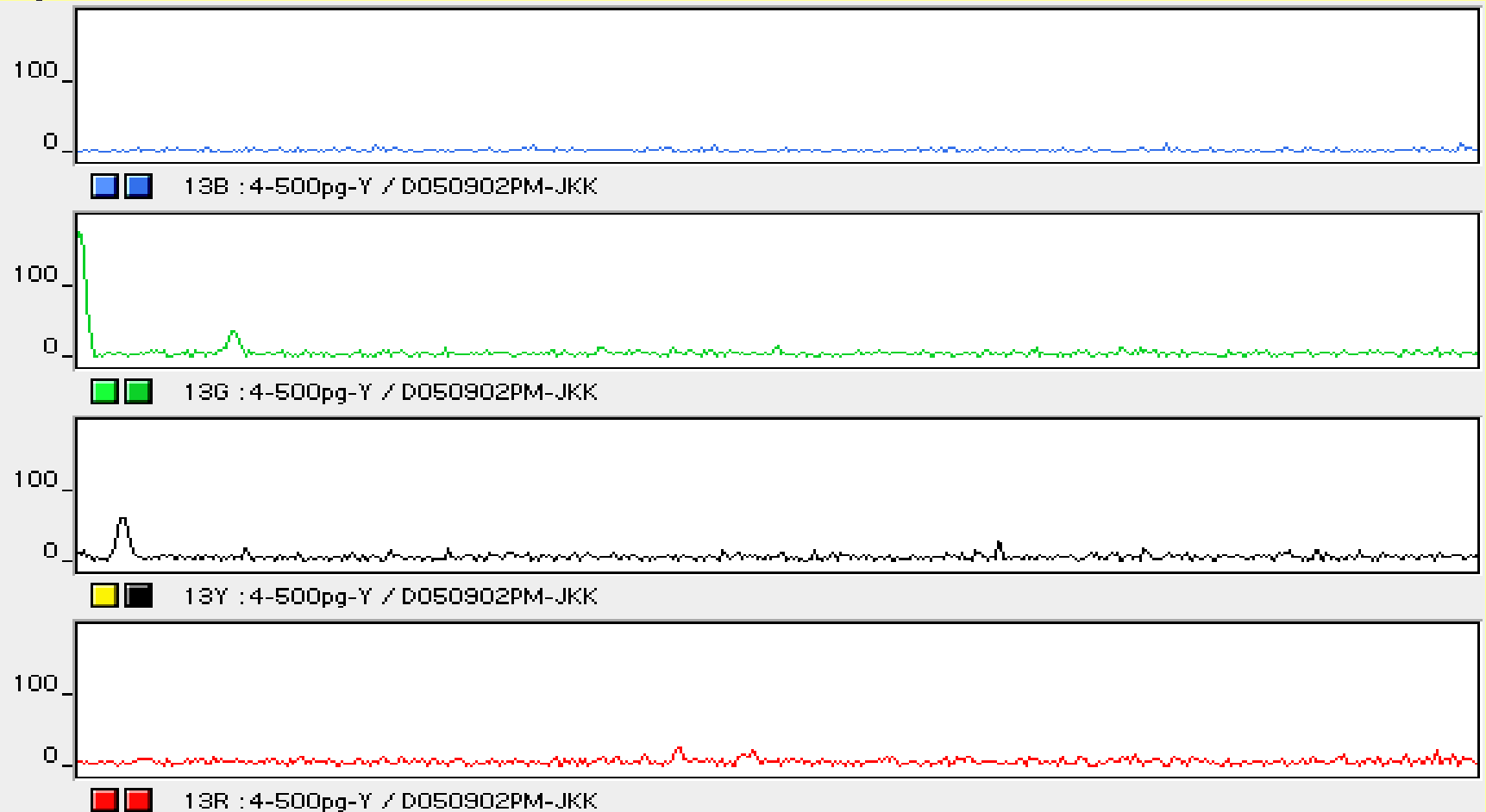


# Male:Female Mixtures II

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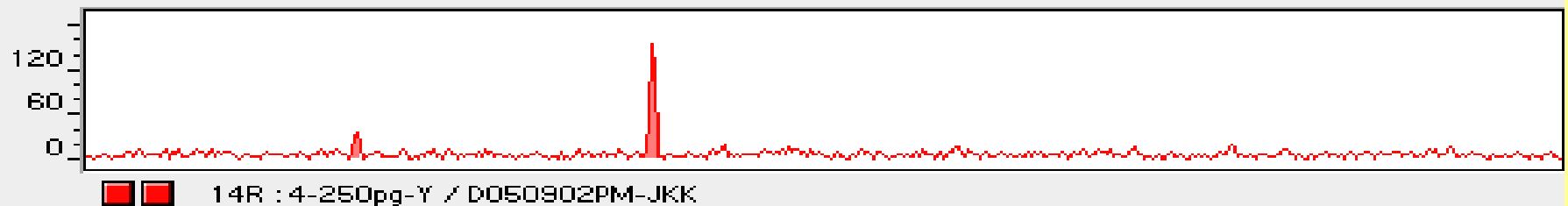
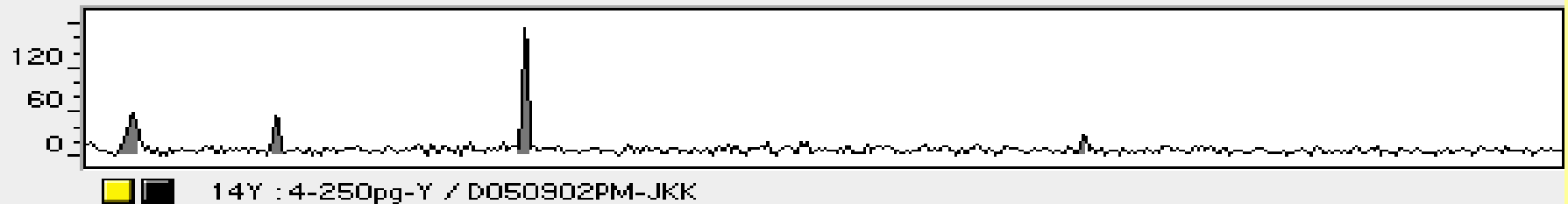
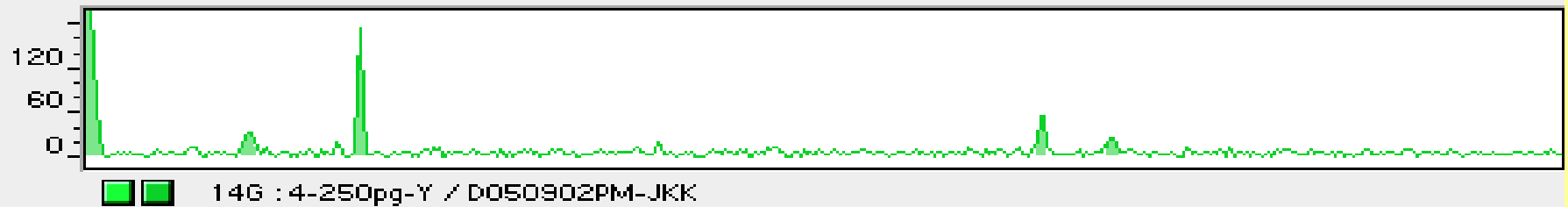
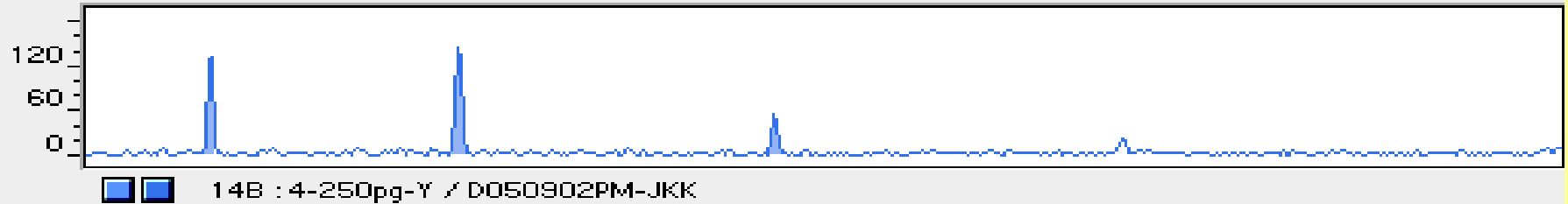
- Prepared 1:5000 and 1:10,000 mixtures
  - male:female DNA
  
- Amplified 125, 250 & 500pg male DNA
  - 625, 1250 & 2500ng female DNA
  - 1250, 2500 & 5000ng female DNA

# Male:Female Mixtures II

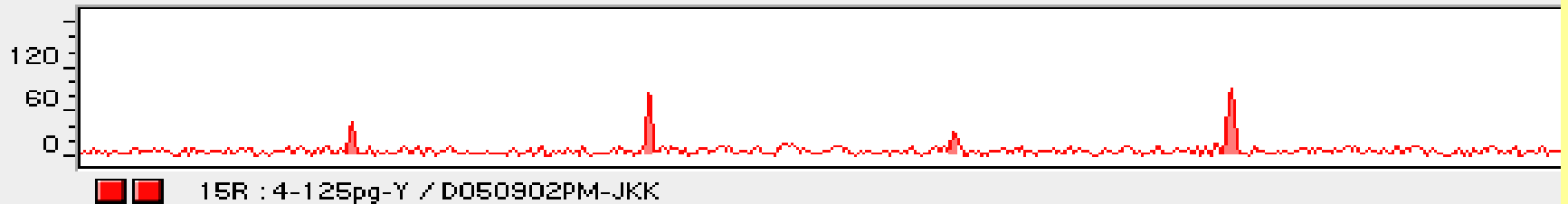
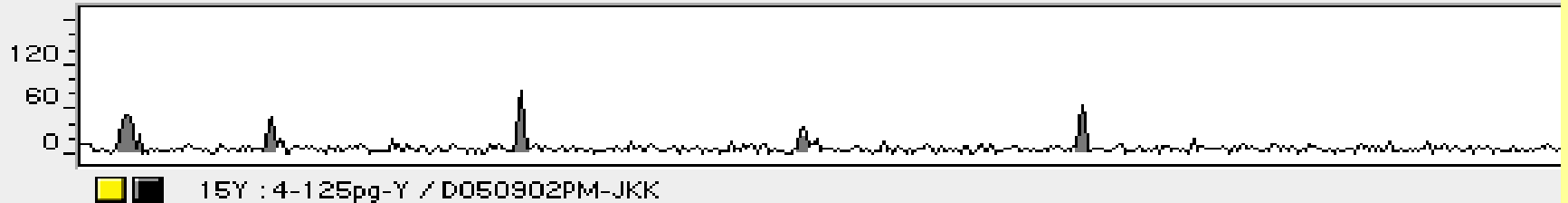
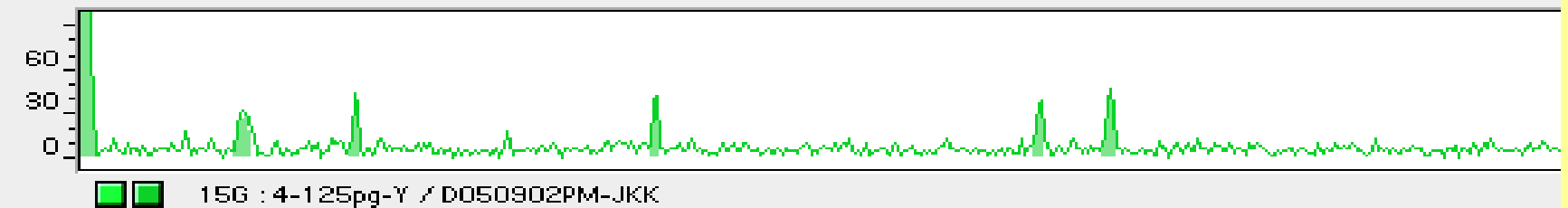
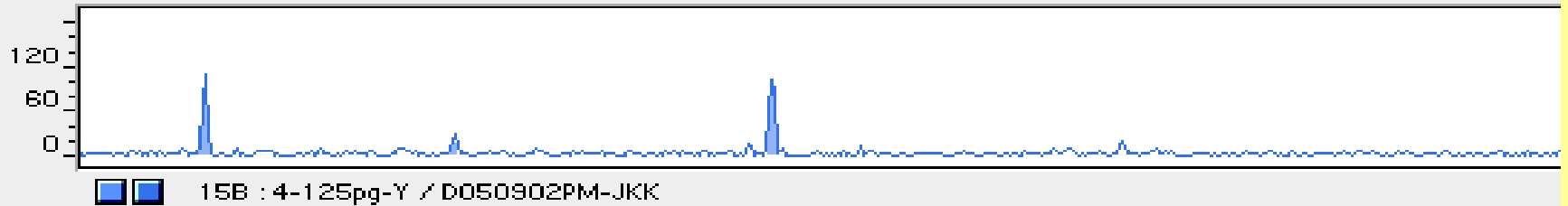




# Male:Female Mixtures II



# Male:Female Mixtures II





# Yfiler Adjudicated Cases

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- 12 cases
- All samples that showed any sign of sufficient male DNA (autosomal profile, weak Y at amelogenin, quantifiable male DNA using Quantifiler Y) gave a Yfiler profile
- 14 “female fractions”
  - 7 complete profiles
  - 5 mixtures with predominant profile
  - 1 partial profile
  - 1 no results (Quantifiler Y indicated no male DNA)



# Yfiler Adjudicated Cases cont.

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- Only other sample that didn't yield a profile had only 16pg of male DNA
- 8 amylase positive samples tested
  - Autosomal results varied between matching suspect, mixture of V & S, or no male DNA detected
  - Yfiler profiles obtained for all samples



# What we learned from validation....

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- Optimal input is 500-800pg male DNA (based on Quantifiler Y)
- No need to amp Female knowns
- Ratio of male:female DNA is important to help determine amount of male DNA to amplify
- Beneficial to try varying amount of input male DNA when an overwhelming amount of female DNA is present



# What we learned from validation....

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- Non-sperm cell fractions are a great source of male DNA.
- Y-STR testing may be appropriate in cases where victim was raped by multiple assailants, but not all assailants are detected with autosomal testing



# Case Acceptance

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- Positive for semen or amylase with no male component detected by autosomal testing
  - Low number of sperm
  - Aspermatics - positive p30
  - Amylase positive vaginal/perineal swabs
- Fingernail scrapings/swabbings
- Criminal paternity where fetal tissue of male offspring cannot be distinguished from maternal tissue
- Mixtures (weak male types)



# Casework Statistics

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- 31 cases reported
- Types of cases
  - CSC; Homicide; Death Investigation
- Types of samples
  - Fingernail scrapings
  - Semen identified on various items
  - Amylase indicated on various items
- 52 question samples





# Casework Statistics

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## 23 Y Profiles

matches suspect	14
suspect excluded	7
matches suspect others excluded	2

## 13 Partial Y Profiles

matches suspect	2
mixture	7
no interpretation	4

## 7 Mixtures

pred profile matches suspect	4
no interpretation	3

## 9 No Y Profile

no male DNA present	6
male DNA present, no results	3



# Court Experience

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- Evidentiary hearing in Hennepin County
  - Judge ruled YSTRs acceptable with appropriate statistics
- Presented in court in five cases



# Reporting Results

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- Qualitative statement
- Database search
- Quantitative statement
  - statistical significance of a profile



## Reporting Results:

### Qualitative statement for Exclusion

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“The Y-chromosomal DNA profile obtained from Item 1 does not match the Y-chromosomal DNA profile obtained from the known sample of John Doe. Therefore, John Doe is excluded as the contributor of this male DNA.”



## Reporting Results:

### Qualitative statement for Inclusion

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“The Y-chromosomal DNA profile obtained from Item 1 matches the Y-chromosomal DNA profile obtained from the known sample of John Doe. Therefore, neither John Doe nor any of his paternally related male relatives can be excluded as the contributor of this male DNA.”



# Population Database

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- ABI Database – [www.appliedbiosystems.com](http://www.appliedbiosystems.com)
  - international database
  - ~3500 population samples
  - 8 population subgroups



# Population Databases

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Don't assume that the available databases have different individuals. Many smaller population databases are represented in several databases.



# Applied Biosystems Database

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Population	# Haplotypes	# Haplotypes (with Selected Alleles)	Frequency
African American	985	<u>1</u>	0.0010
Asian	330	0	0
Caucasian	1276	0	0
Filipino	105	0	0
Hispanic	597	0	0
Native American	106	0	0
Sub-saharan African	59	0	0
Vietnamese	103	0	0
<b>All</b>	<b>3561</b>	<b><u>1</u></b>	<b>0.0003</b>





# Statistical Significance of a match

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- Size of database for each population group
- Number of observations seen in each population group
- 95% upper limit frequency



# 95% confidence limit

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- If the haplotype has been observed in the database:

$$p + 1.96 \sqrt{\frac{(p)(1-p)}{n}}$$

Where:  $p = x/n$   
 $x$  = number of observations  
 $n$  = size of database



# 95% confidence limit

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- If the haplotype has not been observed in the database:

$$1 - (0.05)^{1/n}$$

**Where: n = size of database**



# Quantitative statement

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The Y-chromosomal profile obtained from Item 1 has been observed in the population groups as follows:

<b>Population</b>	<b># observations</b>	<b>Database size</b>	<b>Upper Limit Frequency (%)</b>
<b>Caucasian</b>	<b>0</b>	<b>1276</b>	<b>0.234</b>
<b>African American</b>	<b>0</b>	<b>985</b>	<b>0.304</b>
<b>Hispanic</b>	<b>1</b>	<b>597</b>	<b>0.496</b>

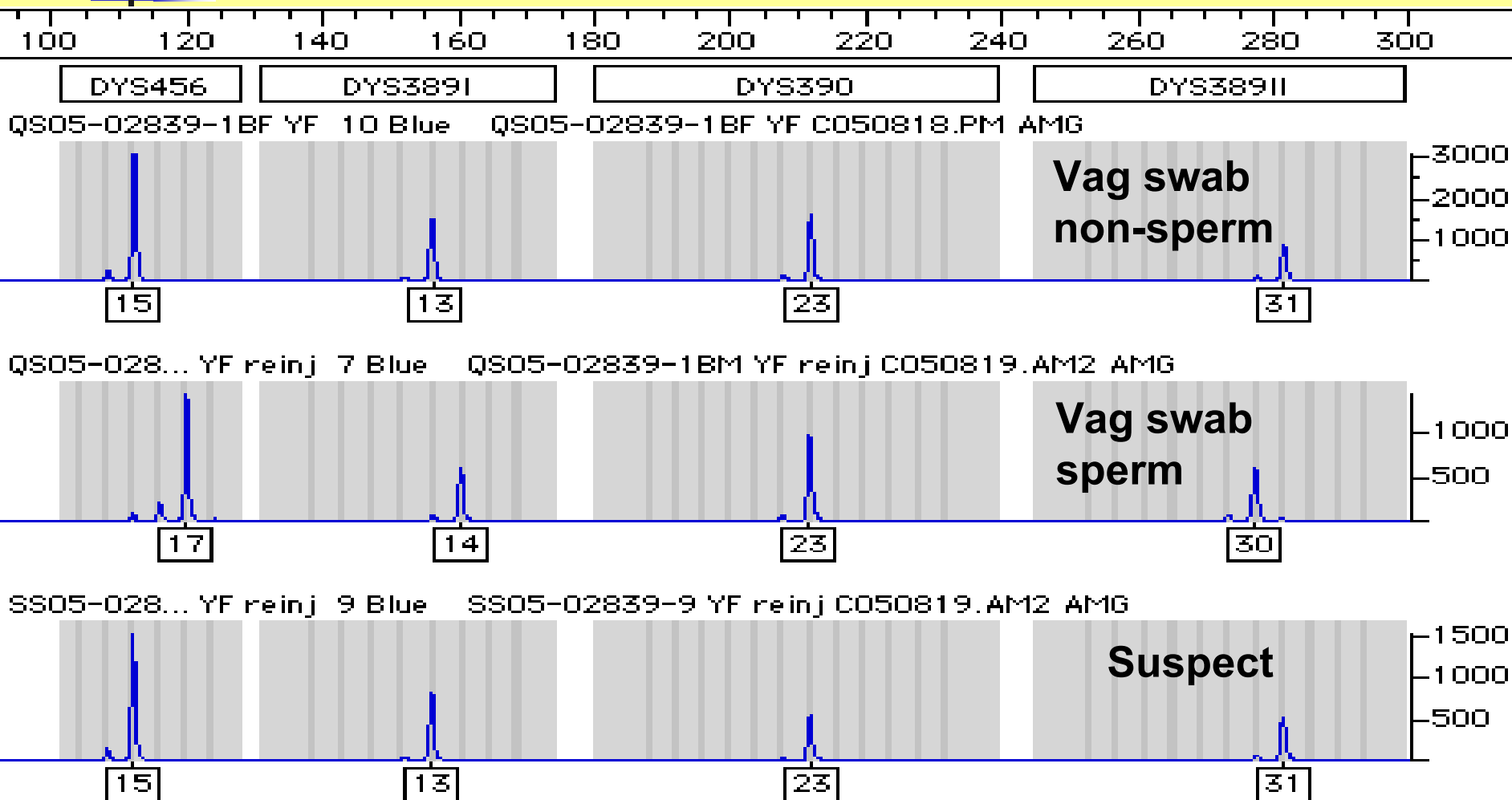


# Case Example 1

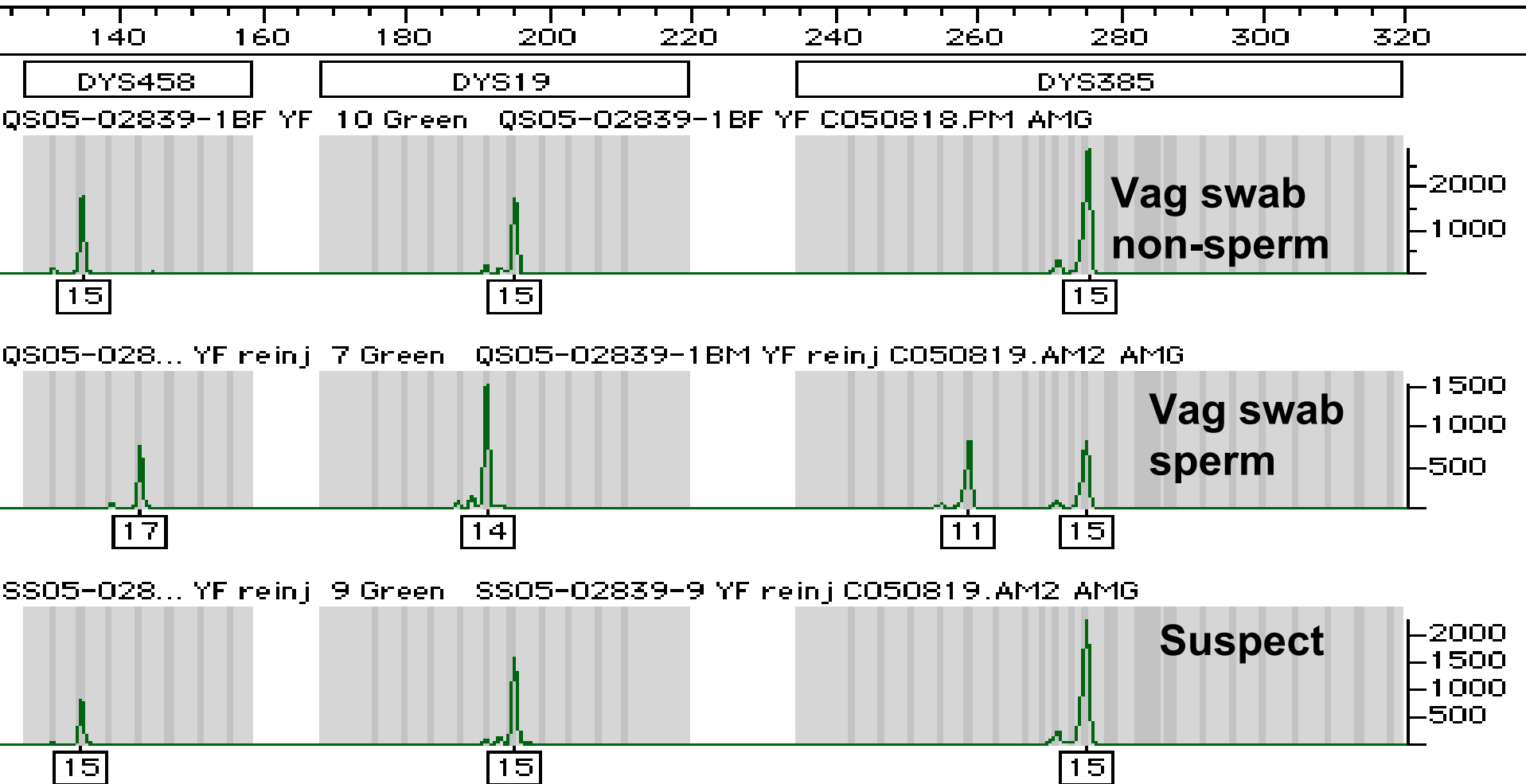
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- CSC Case
- Amylase indicated on vag swabs & underwear
- Semen identified on vag swabs & underwear
- Autosomal results → no DNA types unlike those obtained from the victim

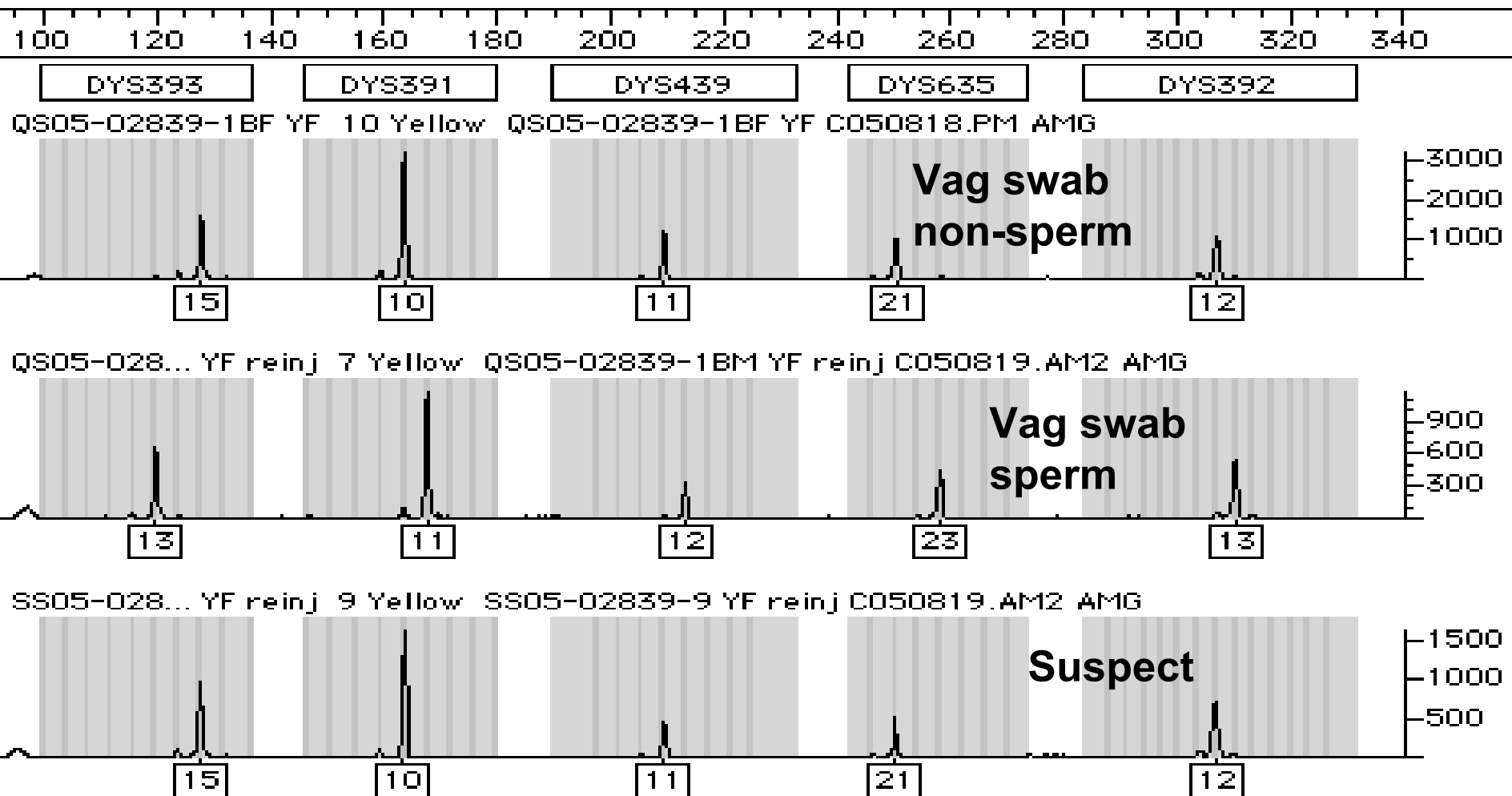
# Case Example 1



# Case Example 1

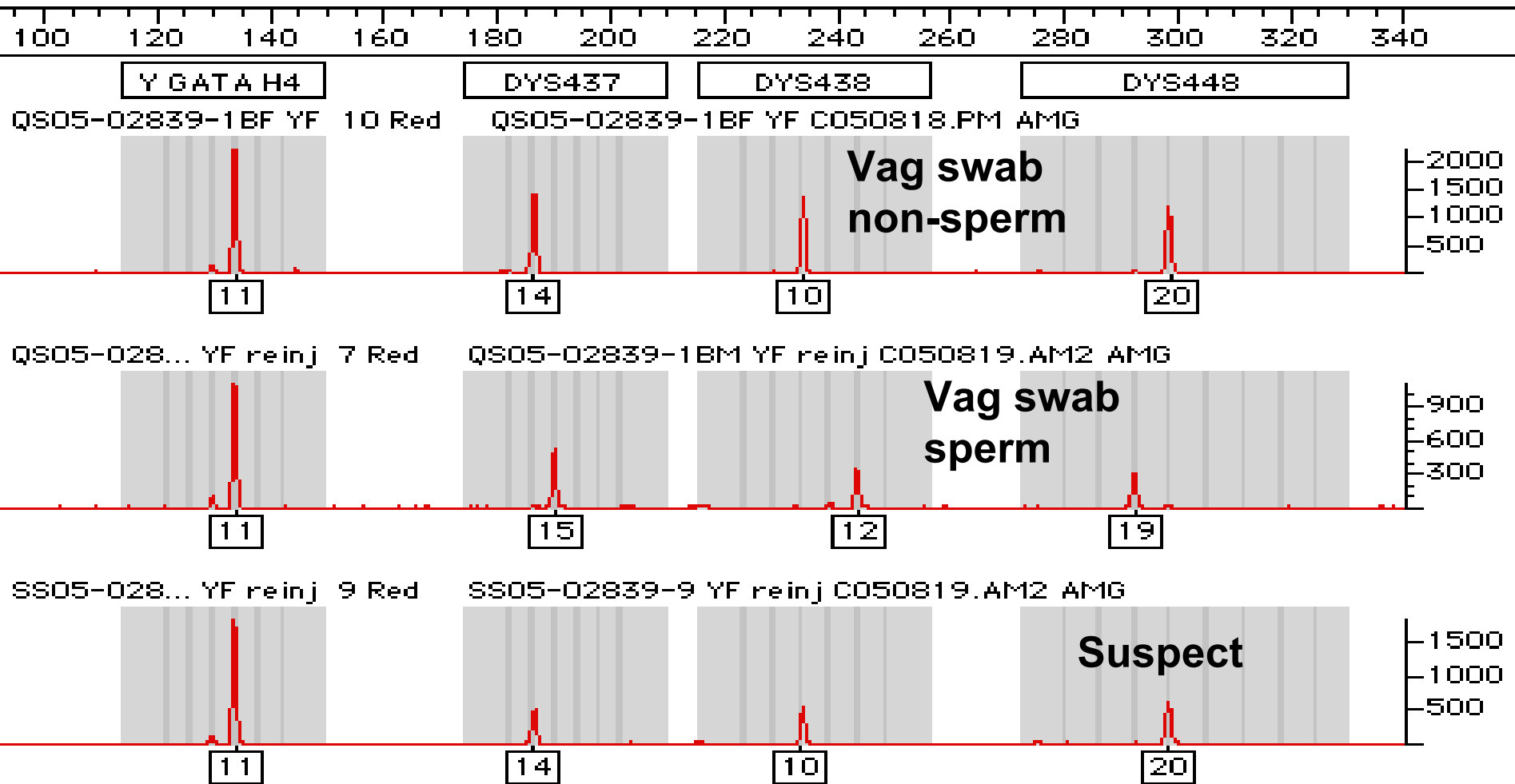


# Case Example 1





# Case Example 1



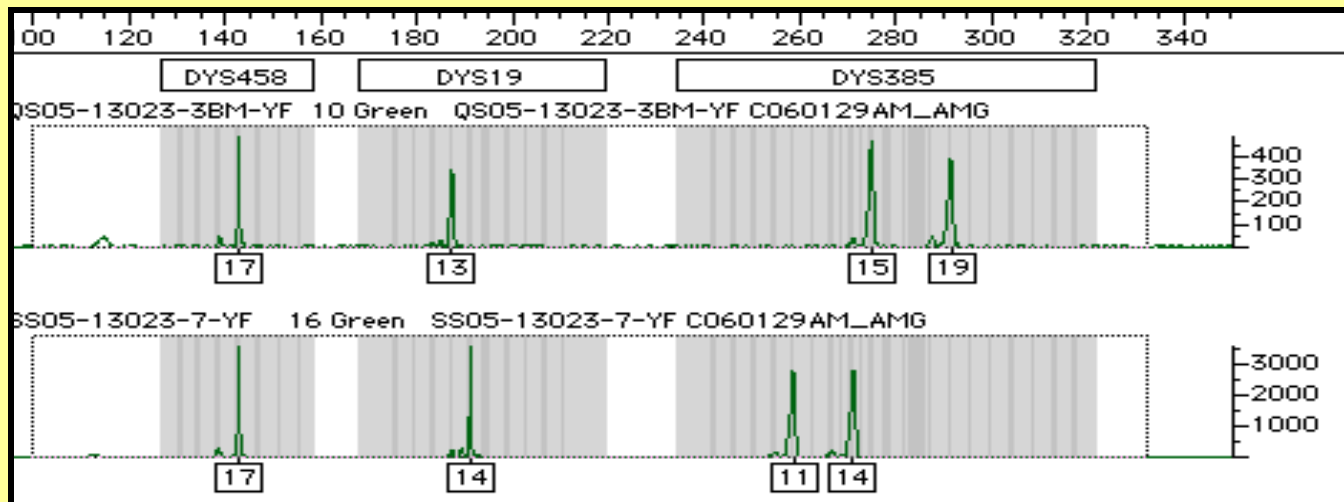
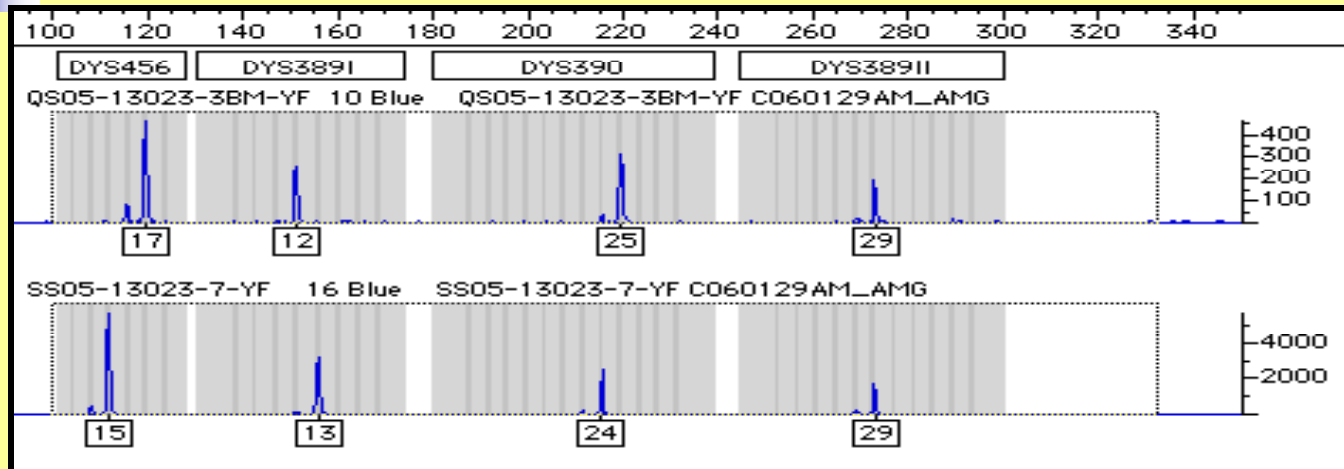


## Case Example 2

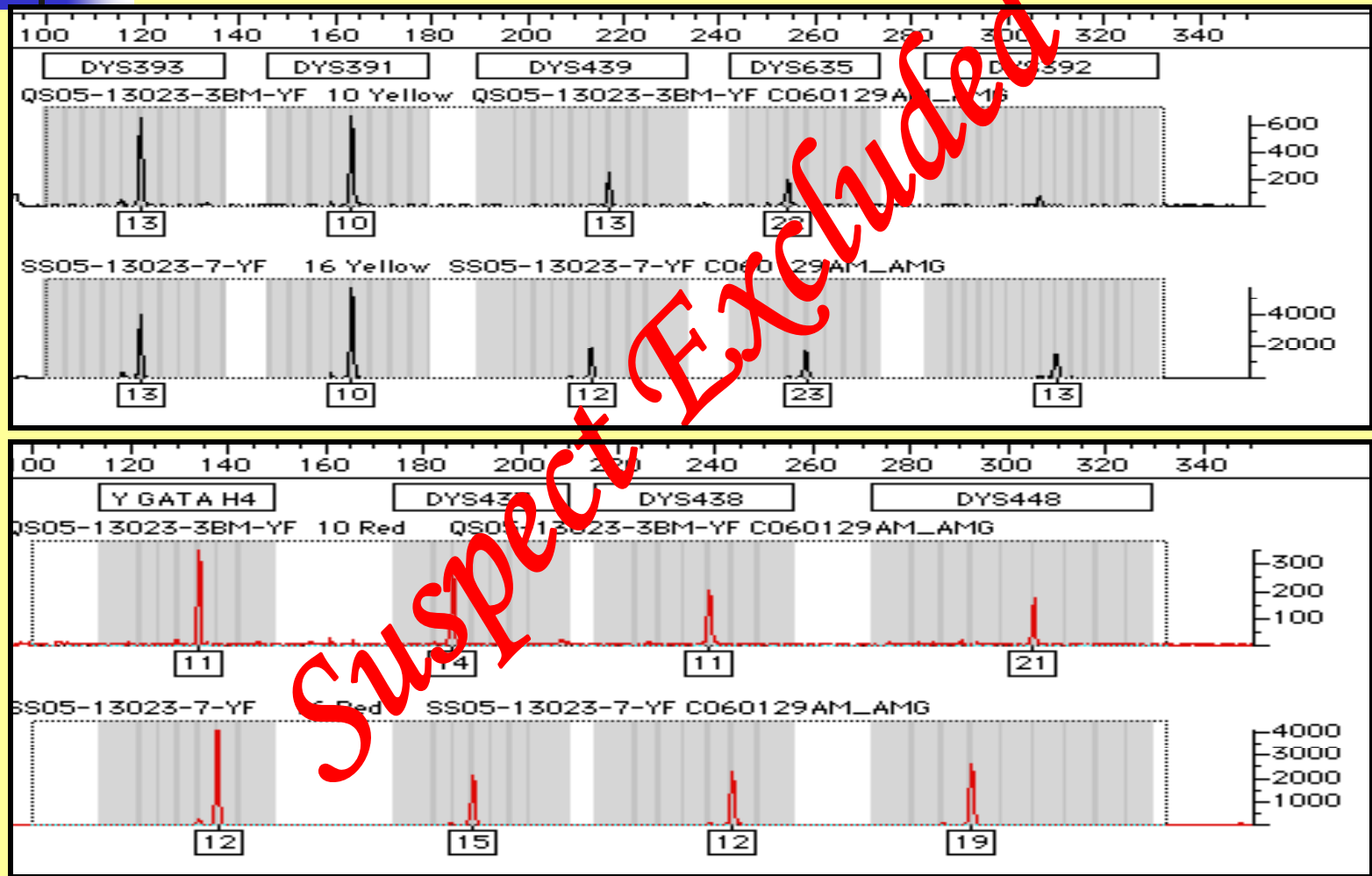
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- CSC case
- Semen identified on vaginal swab
- Autosomal – no DNA types unlike those from the victim

# Case Example 2



# Case Example 2



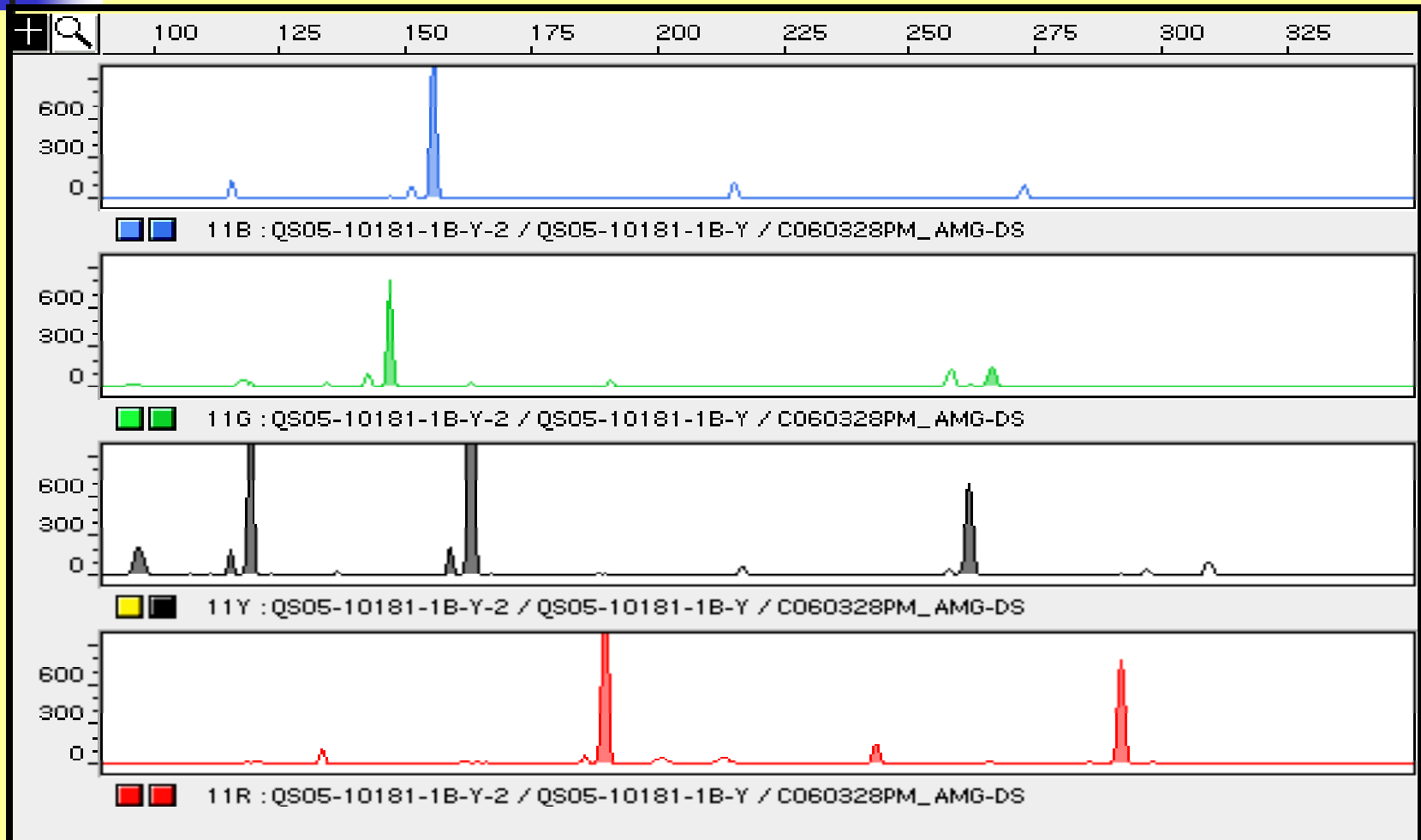


# Case Example 3

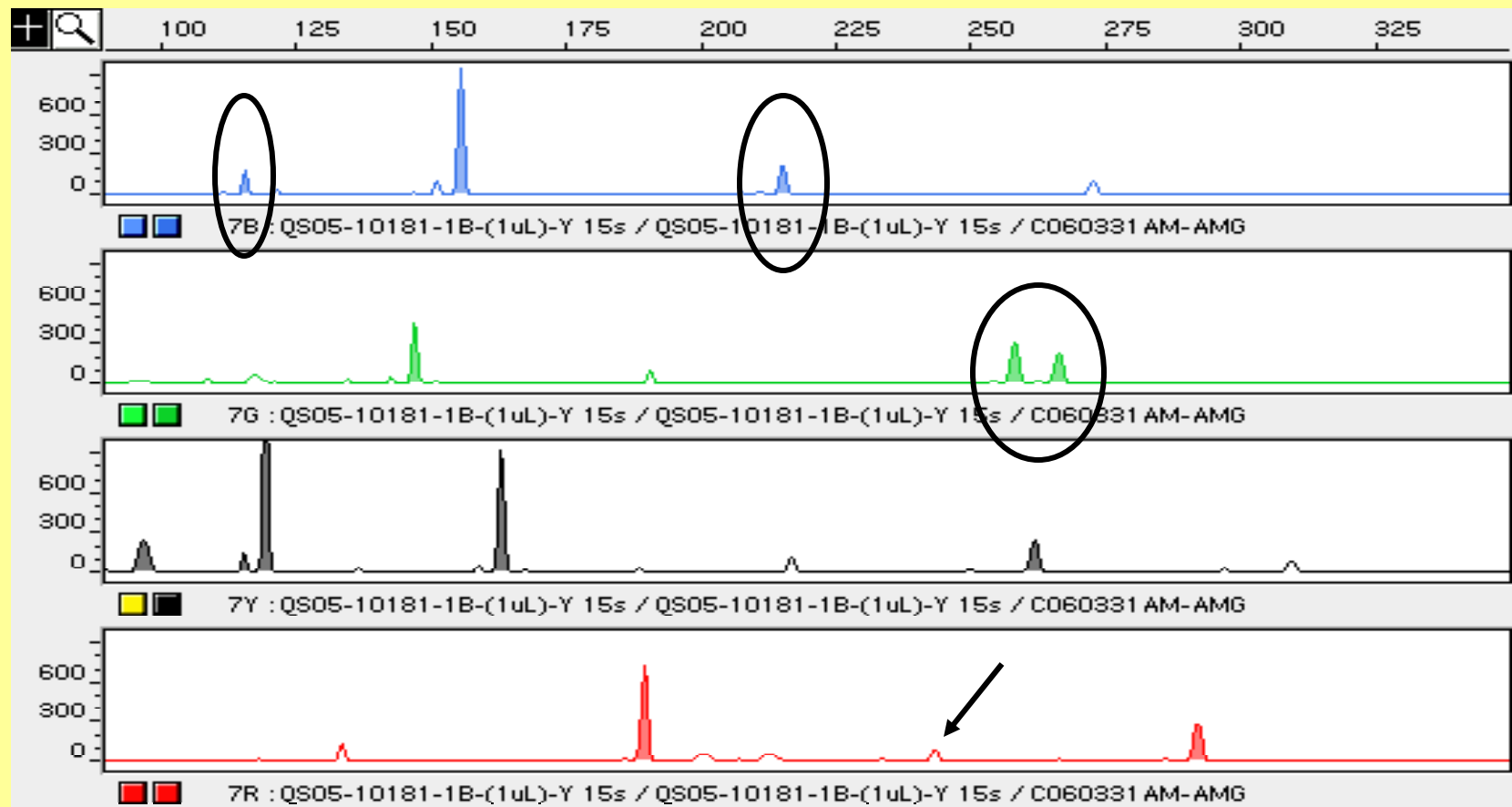
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- CSC Case
- Amylase indicated on vag swabs (1B) and perineal swabs (1C)
- Quant Human:Quant Y
  - 1B 2500:1 (50ng/uL to 0.02ng/uL)
  - 1C no male DNA detected

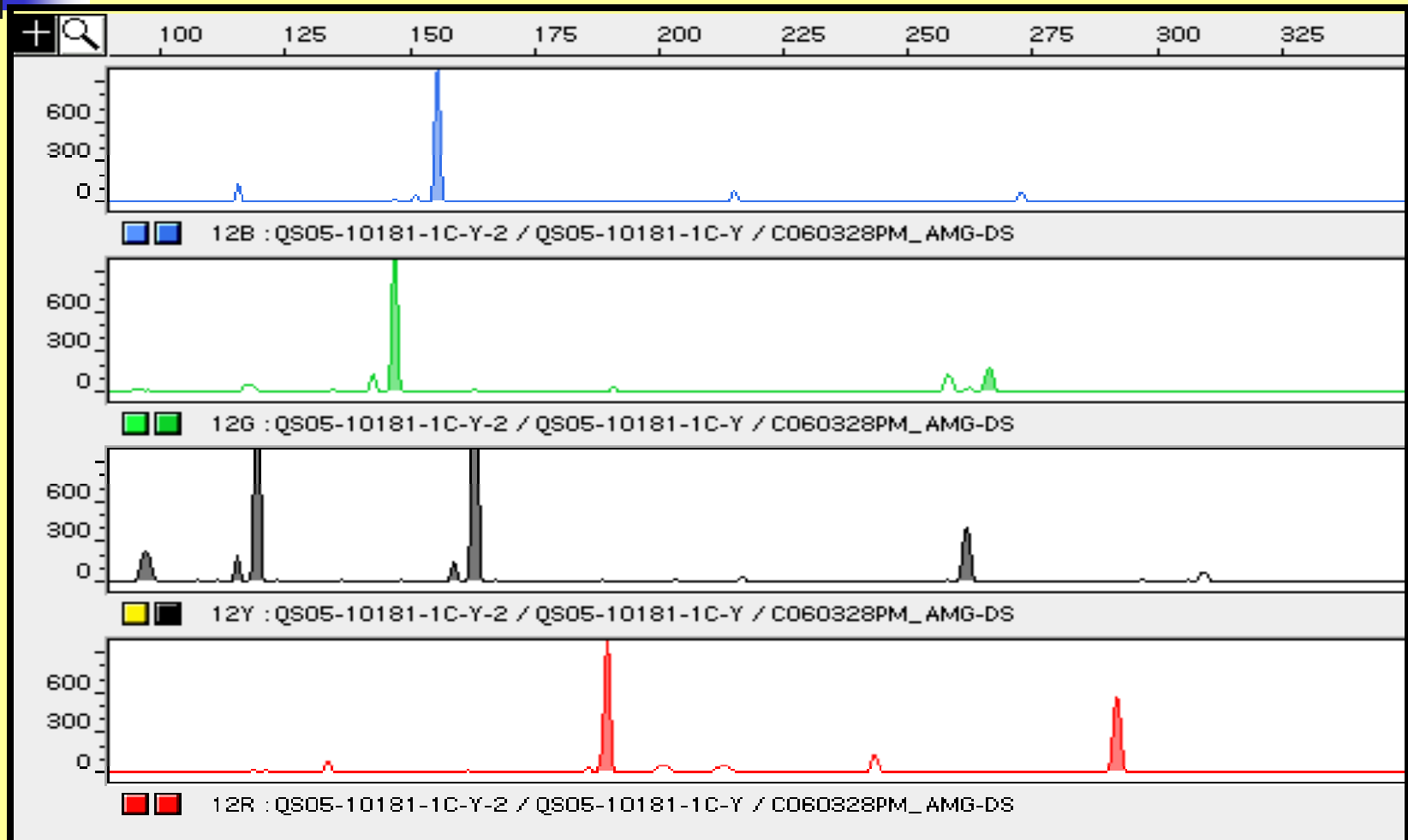
# Case Example 3



# Case Example 3 -- Results



# Case Example 3 -- Results







# What we learned from casework

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- Testing for amylase on vaginal swabs is now done routinely
- Non-sperm cell fractions from differential extractions are an excellent source of male DNA for YSTR testing
- Quantifiler Y negative doesn't necessarily mean no Yfiler results will be obtained



# What we've learned from casework

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*This kit rocks!!!!*





# Thanks.....

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- BCA Scientists

- Amy Liberty
- Trina Kuriger
- Megan Ulland

- ABI

- Lisa Calandro



# Contact Info

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