

ORCHID CELLMARK

A Leader in Identity DNA Testing Services

Y-STR Comparative Study and Interesting Case Examples

Cassie L. Johnson, M.S.

Forensic DNA Analyst IV

Technical Leader of Y-STR and Mitochondrial DNA Analysis

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Applied Biosystems Human Identification University

Future Trends in Forensic DNA Technology



The Good and the Bad of the Y-Chromosome



◆ Benefits:

- ❖ Paternal inheritance → male specific – female DNA does not amplify
- ❖ Haploid profile (1 peak per genetic marker) can make interpretation fairly easy

◆ Drawbacks:

- ❖ Y markers are inherited as a group – no independent assortment
 - ◆ Since the Y markers are all linked, they are treated as a single unit
 - ◆ Product rule cannot be applied
 - ◆ Must use the counting method – **the frequency depends entirely on the size of the Y-STR database**
- ❖ Can't discriminate between patrilineal relatives

Y-STR Timeline

2001

2002

2003

2004

2005



Few labs offering Y-STR testing
mainly home-brews
Y-STRs fairly new for forensic casework
mostly used in relatedness cases?
were home-brews designed for compromised samples?
Unfamiliar to law enforcement & attorneys

When are Ys Used in Forensic Casework?



- ◆ Autosomal testing yields little or no male DNA profile
 - ❖ Samples where female DNA is in vast excess of male DNA
 - ❖ Can make interpretation tricky or impossible
- ◆ Very few or no sperm are detected
- ◆ Differential extraction is not possible
- ◆ Clarify the number of male contributors
- ◆ “Last resort” type of testing:
 - ❖ Cold cases
 - ❖ Post-conviction cases

What sample types have been tested with Y-STRs?

- 
- ◆ Vaginal swabs/washes
 - ◆ Anal swabs
 - ◆ Oral swabs
 - ◆ Fingernail clippings
 - ◆ Toenails
 - ◆ Ligatures (twine, shoelace, pantyhose, vacuum cleaner cord)
 - ◆ Guns
 - ◆ Bullets/cartridges
 - ◆ Panties
 - ◆ Feminine products
 - ◆ Swabs from explosive devices
 - ◆ Skillet
 - ◆ Condoms
 - ◆ Hair root
 - ◆ Swabbing from bottle
 - ◆ Tape
 - ◆ Airbag
 - ◆ Razor contents
 - ◆ Swabbings from skin (ex: neck, breast)
 - ◆ Cigarette butts
 - ◆ Bone
 - ◆ Diaper
 - ◆ Kleenex
 - ◆ Towels, washcloths
 - ◆ Vaginal slides/smears
 - ◆ Bedsheets
 - ◆ Fetal tissue
 - ◆ Cuttings from clothes (ex: shirt)
 - ◆ External genital swabs
 - ◆ DNA extracts from other labs

Y-STR Multiplexes



- ◆ Large increase in the amount of Y-STR testing used in forensic cases
- ◆ Which kit works the best?
 - ❖ Powerplex Y?
 - ❖ Yfiler?
 - ❖ 10-Plex?

The Game Plan



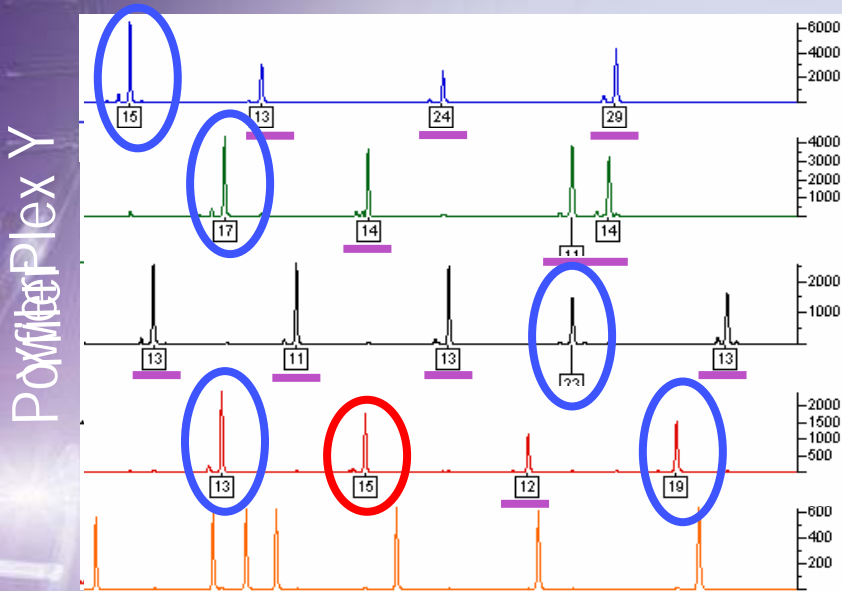
- ◆ Each system had been internally validated
 - ❖ 2002 (10-plex)
 - ❖ 2004 (PowerPlex Y)
 - ❖ 2005 (Yfiler)
- ... **independently**
- ◆ Eliminate as many variables as possible to get a head-to-head comparison of the multiplexes
 - ❖ Same DNA extracts
 - ❖ Same thermocycler
 - ❖ Same 3100
 - ❖ Little time difference
- ◆ Today's talk will focus on PowerPlex Y and Yfiler

What Criteria Should be Examined?



- ◆ Effect of female DNA
 - ❖ Does female DNA interfere with detection of male DNA?
 - ❖ How much female DNA can be present and still yield a male profile?
- ◆ Sensitivity
 - ❖ What is the least amount of male DNA that can be detected?
- ◆ Reproducibility
 - ❖ How reproducible is the multiplex in yielding a balanced profile with consistent RFUs?
- ◆ Mixtures of 2 males
 - ❖ Can a mixture of 2 males be distinguished?
- ◆ Marker selection
- ◆ Statistics
- ◆ Overall quality of the electropherograms

Marker Selection



All U.S. Haplotype markers

++

DYS437
DYS437

12 total markers
+
5 additional markers

17 total markers

U.S. Haplotype

	PowerPlex Y	Yfiler
DYS391	*	*
DYS389 I/II	*	*
DYS439	*	*
DYS438	*	*
DYS385a/b	*	*
DYS19	*	*
DYS392	*	*
DYS393	*	*
DYS390	*	*
DYS437	*	*
DYS456		*
DYS458		*
DYS635		*
H4		*
DYS448		*

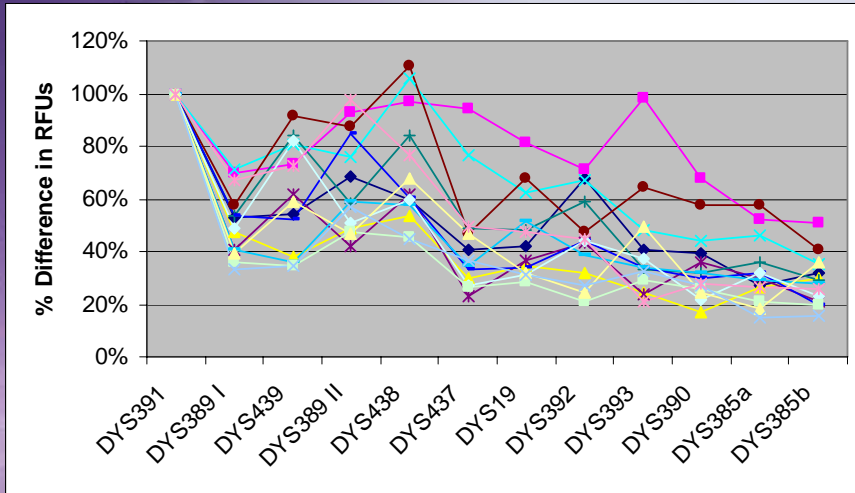
Reproducibility



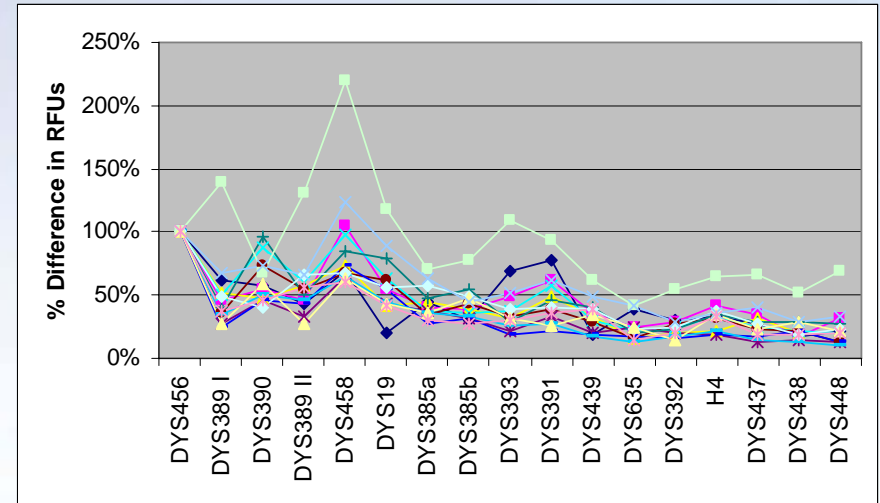
- ◆ Positive control amplified 14 times
- ◆ Is the balance of markers consistent from amp to amp?
- ◆ Are the RFUs consistent from sample to sample?

Reproducibility

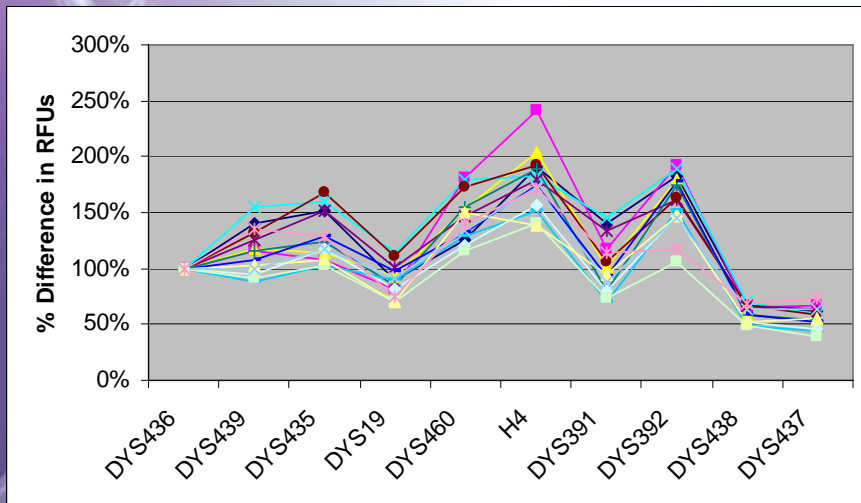
PowerPlex Y



Yfiler



10-Plex



n = 14	Yfiler	PowerPlex Y	10-Plex
Ave. RFU	2118 +/- 41%	2868 +/- 52%	3273 +/- 25%

Loci: Highest \longrightarrow Lowest

Ave RFU: Lowest \longleftarrow Highest

Sensitivity Studies

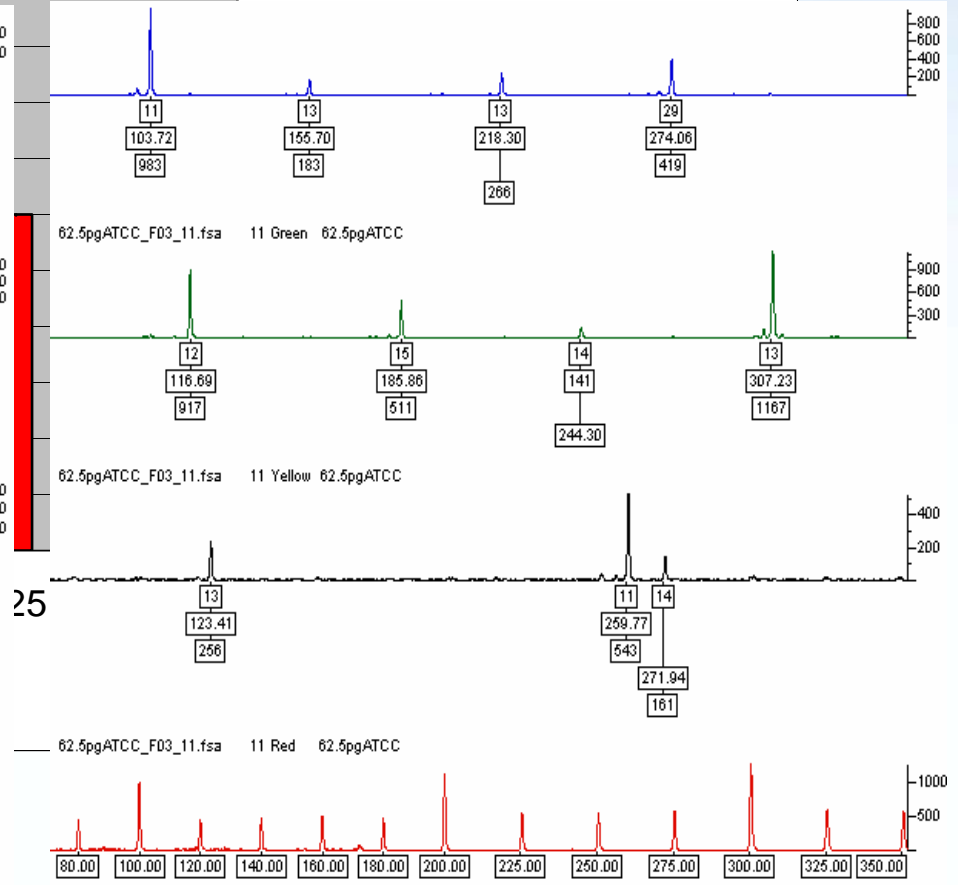
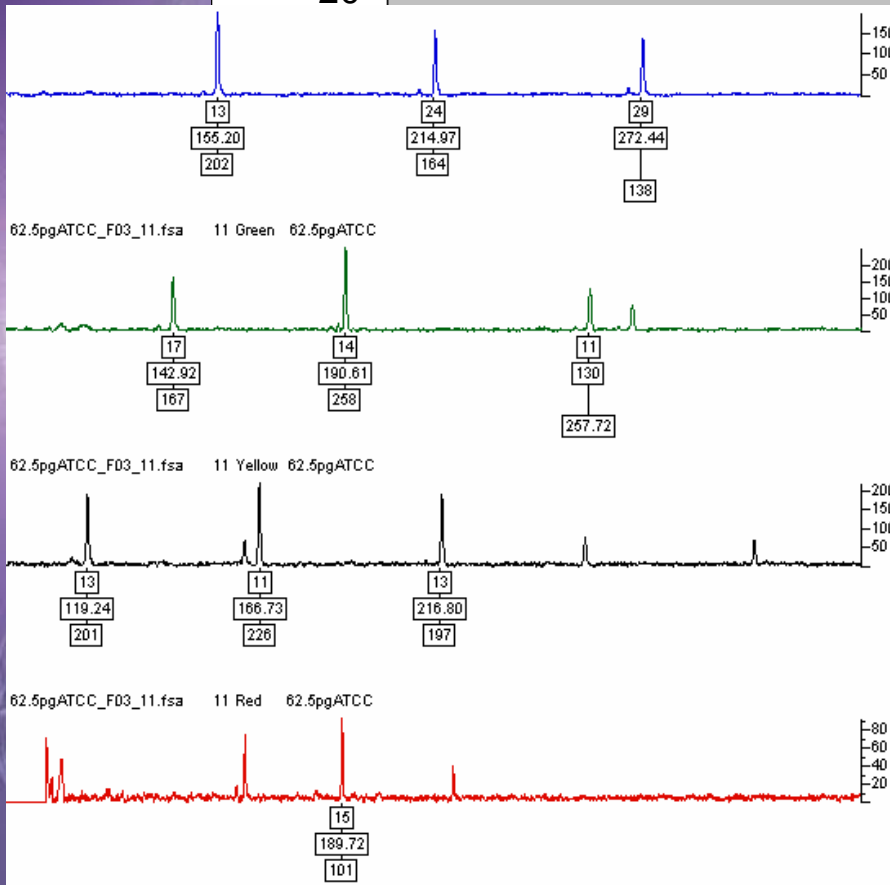


- ◆ Varied input DNA from 15.63 pg – 4 ng
- ◆ Amplified positive control 5 times at each quantity
- ◆ What is the least amount of DNA that can be amplified and still yield a full Y-STR profile?

Sensitivity Studies

20 Yfiler

PowerPlex Y



25

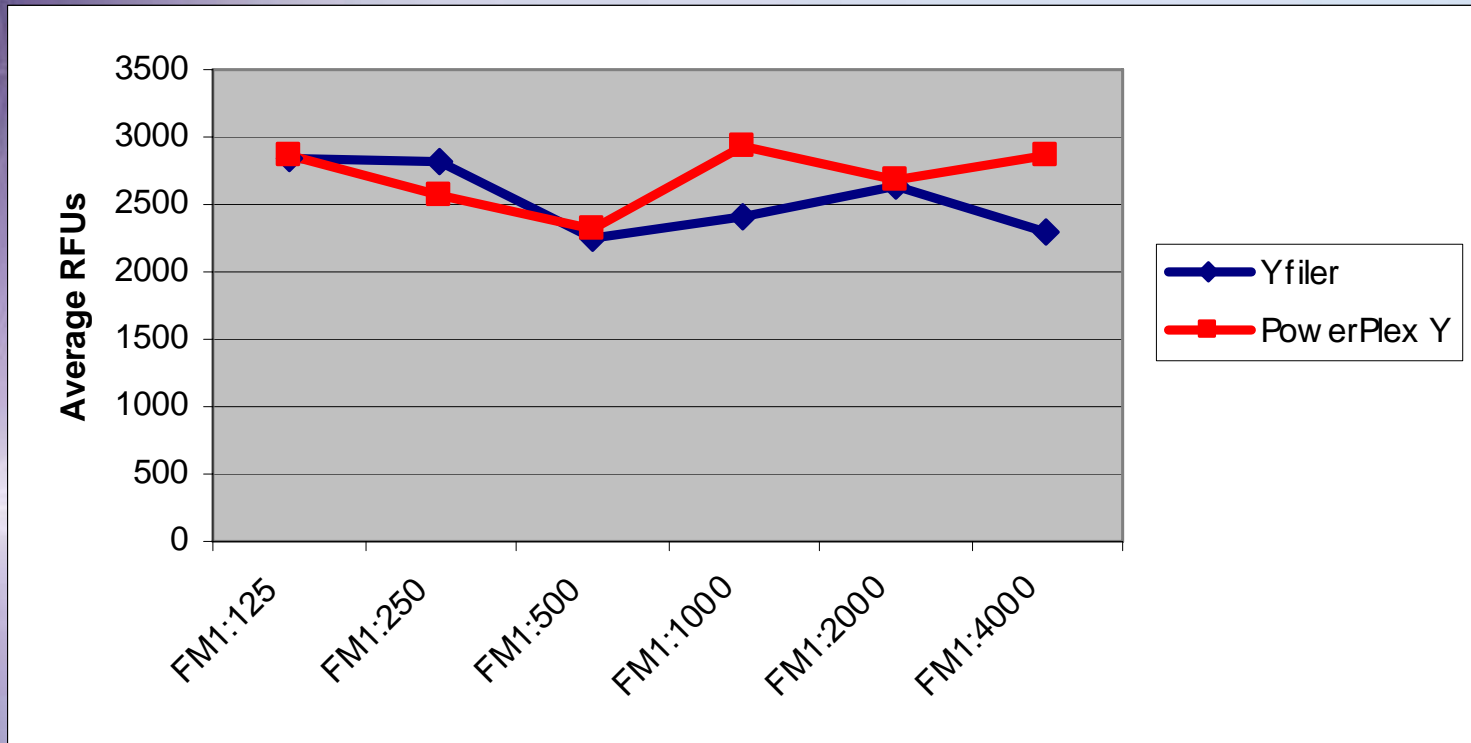
62.5 pg input DNA

Female:Male Mixtures



- ◆ Male specificity is extremely important!
- ◆ Forensic samples submitted for Y-STR testing:
 - ❖ Example: vaginal swab
 - ❖ Lots of female DNA
 - ❖ Almost always negative for sperm
 - ❖ Minute amounts of male DNA
- ◆ Amplified in triplicate:
 - ❖ Female:male mixtures ranging from 1:125 to 1:4000
- ◆ Can male DNA be amplified in a large excess of female DNA?

Female:Male Mixtures



- ◆ Yfiler and PowerPlex Y
 - ❖ No interference from female DNA
 - ❖ Comparable RFUs at each female:male ratio tested

Male:Male Mixtures



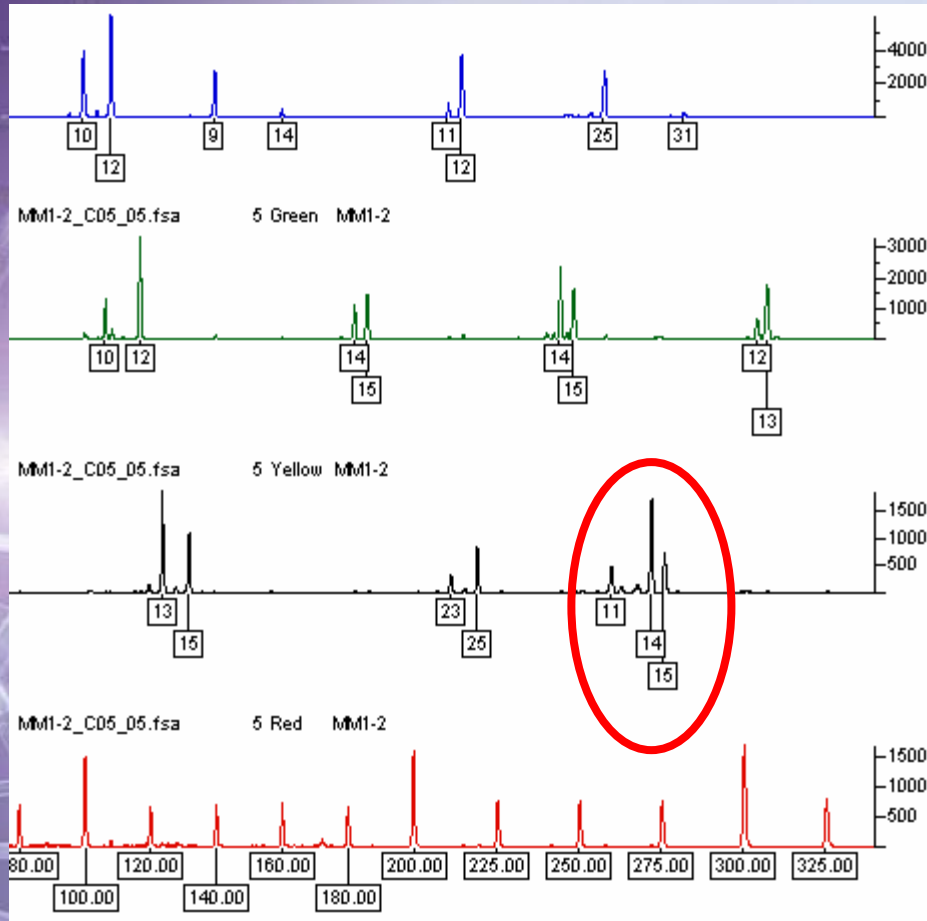
- ◆ Possible scenarios:
 - ❖ Female victim with 2 male assailants
 - ❖ 1 assailant and 1 consensual partner
 - ❖ Male victim and male assailant
- ◆ Can 2 male profiles reliably be deduced based on peak heights?
- ◆ Do the peak height ratios accurately reflect the mixture?
- ◆ Male:male mixtures ranging from 1:1 to 1:10 amplified 4 times each

Male:Male Mixtures

n = 4	PowerPlex Y	Yfiler
Ave. PHR in MM10:1	10.2	7.6
# correct profiles	100%	100%
Ave. PHR in MM5:1	4.4	3.7*
# correct profiles	75%	50%*
Ave. PHR in MM2:1	2.6	2.0
# correct profiles	25%	25%
Ave. PHR in MM1:1	1.6	1.9
# correct profiles	0%	0%

* Only 2 mixtures of 5:1 tested

PowerPlex Y Male:Male Mixture (1:2)



DYS385a/b:

M1: 11, 14

M2: 15

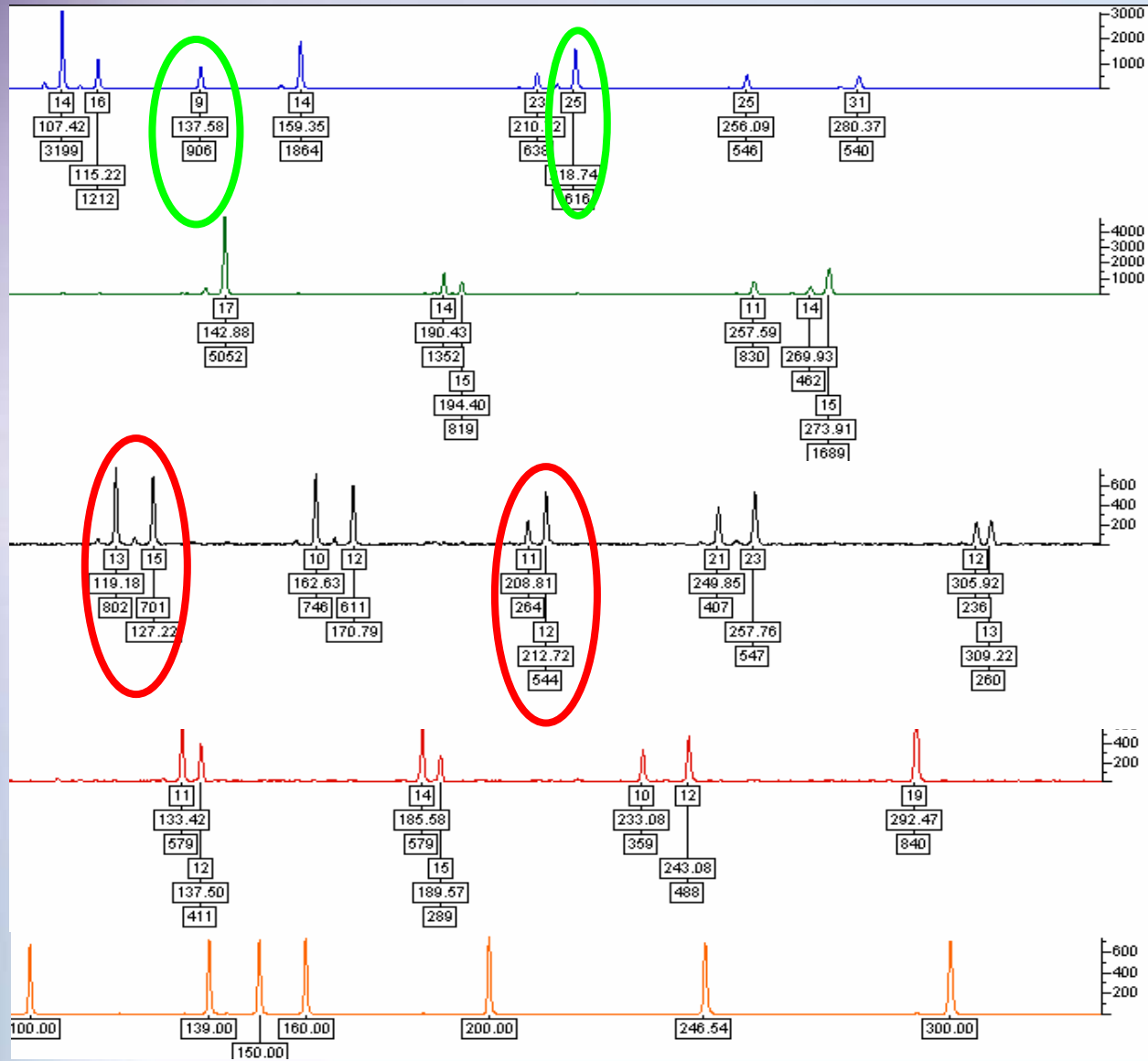
DYS385a/b
interpreted as:

11,15

14

At mixtures closer than 5:1, the correct profiles cannot be deduced

Yfiler Male:Male Mixture (1:1)



Interpretation of Male:Male Mixtures



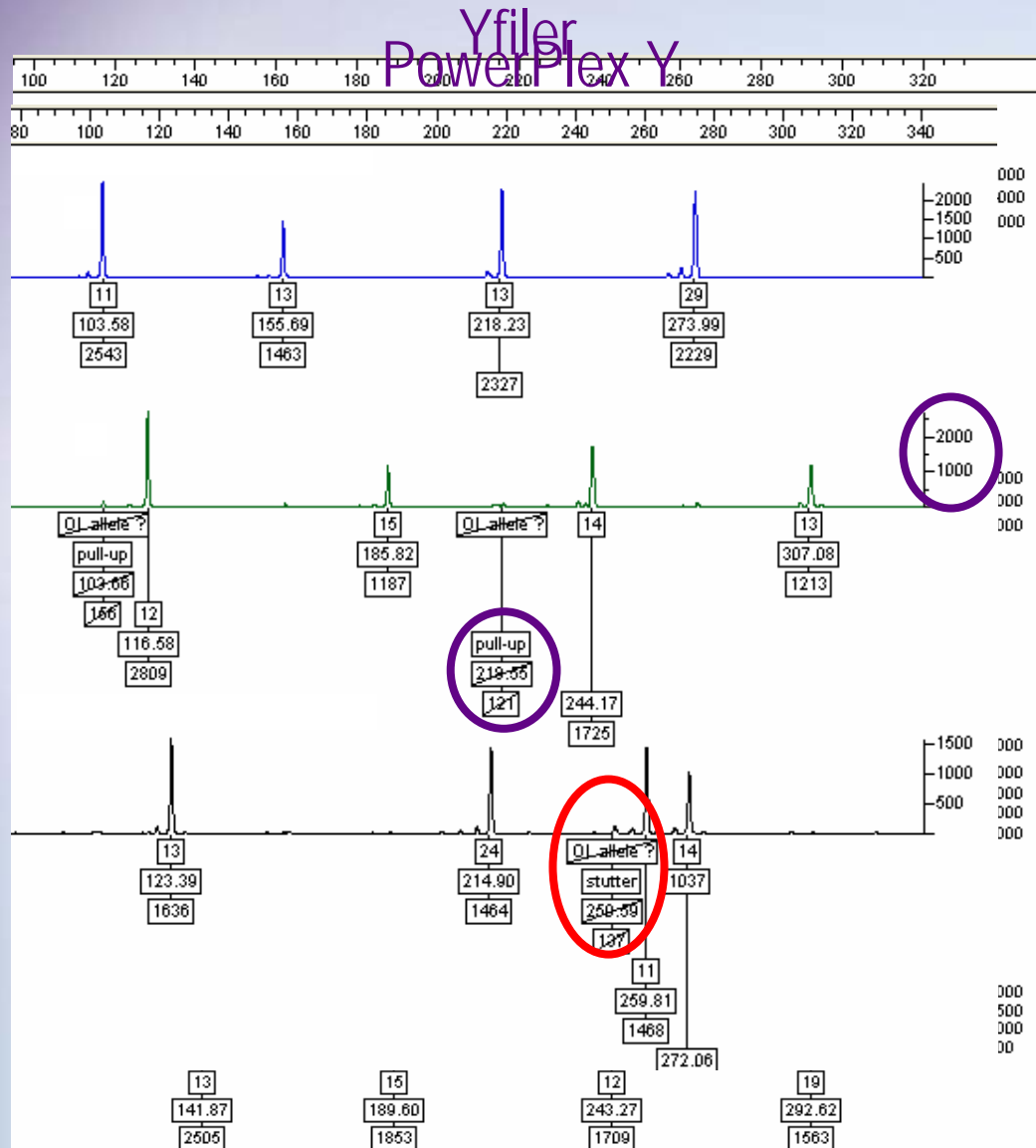
- ◆ Interpret with caution
- ◆ Cannot always deduce with confidence
- ◆ Search for all possible haplotypes and issue statistics accordingly

Y-STR Statistics



- ◆ Statistics are entirely dependent on the size of the database – the bigger the better
- ◆ CURRENT DATABASES (which I'm sure are subject to change):
 - ❖ Promega:
 - ◆ 4004 in 5 populations
 - ❖ Applied Biosystems
 - ◆ 3561 in 8 populations
- ◆ Keep in mind:
 - ❖ ABI's database is currently smaller
 - ❖ HOWEVER: examining an increased number of loci (Yfiler) will increase the likelihood of encountering a unique profile (and therefore better stats!)

Overall Quality of the Electropherograms



Pull-up

Stutter products

Summary Sheet

	PowerPlex Y	Yfiler
Reproducibility: consistent balance with minimal deviation in RFUs of positive control	+	++
Sensitivity: increased sensitivity with minimal male DNA. Acceptable working range of input DNA.	++	+
Male:Male Mixtures: ability to correctly deduce two profiles in a male:male mixture	+	+
Female:Male Mixtures: little/no interference of Y-STR typing in the presence of female DNA	+	+
Degraded DNA: based on the number of markers less than ~200 base pairs in length	+	++
Marker Selection: presence of U.S. Haplotype markers and/or other highly discriminating loci	+	++
Statistics: a larger database increases the statistical power of Y-STRs; possibility of finding unique profile	+	+
Overall E-gram Quality: minimal stutter/pull-up	+	++
	9	12

What Kit is Orchid Cellmark Using?



- ◆ Forensic casework samples:
 - ❖ 1st line of testing: Yfiler
 - ❖ Based on Yfiler results, may proceed with 10-Plex

However...



- ◆ Kit selection should be based on the needs of the lab and/or the case requiring testing
- ◆ Cellmark Dallas has the ability to perform all 3 multiplexes
 - ❖ Allows us to optimize the chances for success in Y-STR typing

Switching gears...

- ◆ a few casework examples using Y-STRs



Case #1: Overwhelming Female DNA



- ◆ Victim ends her cashiering job and walks to her truck in the parking lot.
- ◆ Armed suspect attacks her from behind, forces her into the car, and drives away

The initial attack...

- ◆ 1st drives to a location where the victim's shirt is later found
- ◆ The suspect drives to a nearby trailer house
 - ❖ Sexually assaults the victim
 - ❖ Her DNA is found on the headboard and other items
- ◆ The suspect drives all night and checks into a hotel
 - ❖ Sexually assaults the victim
- ◆ Later that night, the suspect says he will take the victim back home
 - ❖ To this point, she has been compliant

The victim fights back...

- ◆ Suddenly, the suspect turns the truck around
 - ❖ The victim hits him in the face – he loses control
- ◆ The suspect grabs her by the throat, opens the car door, and pushes her out
- ◆ He chokes the victim until she goes limp
- ◆ He goes back to the car and returns with his handgun
- ◆ The suspect pulls her up the embankment and shoots her in the head

The Crime Spree Continues. . .



- ◆ Later that night... suspect robs a convenience store
- ◆ The next morning... suspect attempts a robbery of an RV park
 - ❖ RV park worker shoots the suspect
- ◆ Suspect arrives at a hospital to be treated for his gunshot wound
 - ❖ Recognized by police
- ◆ Victim's body is found in a ditch

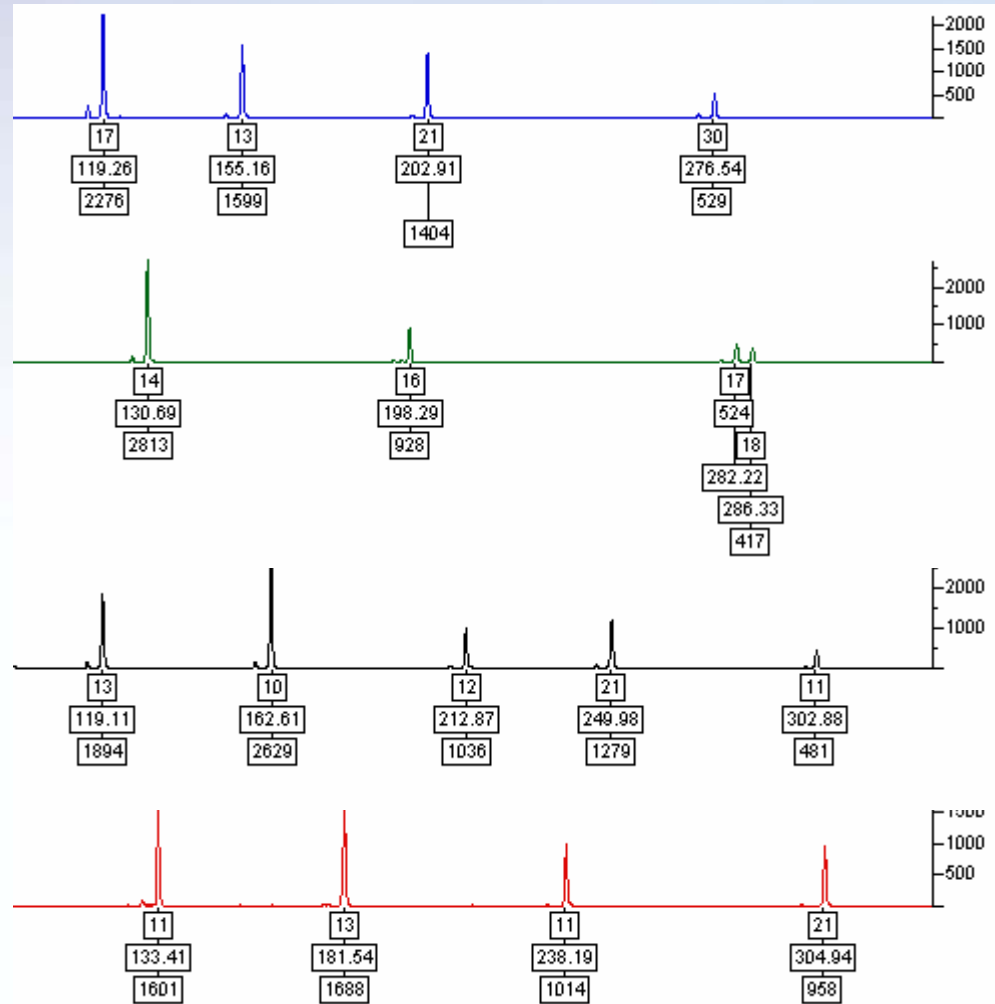
Y-STR testing: overwhelming female DNA



- ◆ Samples from several condoms were submitted for testing
 - ❖ Previous crime lab was unable to get a male profile from the condoms
 - ❖ Presumably too much interference from female DNA
- ◆ Needed to find suspect's DNA to help with the sexual assault charges

Y-STRs on Condom Samples

- ◆ Ran Yfiler
- ◆ Y-STR profile was found both inside and outside of all of the condoms
- ◆ Y-STR profile was consistent with the suspect
- ◆ Database search:
 - ❖ 0 in 3561 males



Sentenced



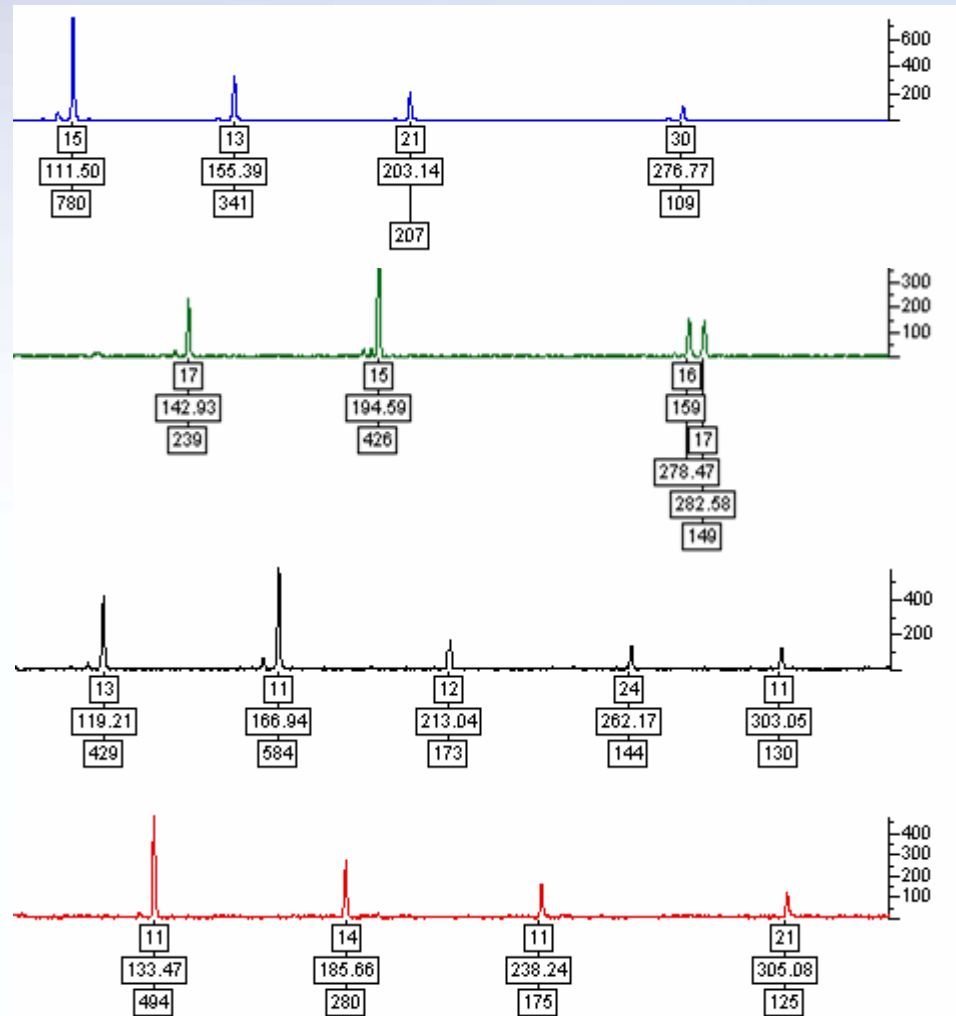
- ◆ Plead guilty to two counts of aggravated sexual assault, aggravated kidnapping, capital murder, and aggravated robbery
- ◆ Sentenced to 5 life terms

Case #2: finding male blood in female blood

- ◆ Differential extraction is not possible
- ◆ Step-father repeatedly stabs his 13 year old step-daughter
- ◆ In an attempt to destroy all evidence, he sets the house on fire
 - ❖ Step-daughter and infant are trapped inside
- ◆ Firefighters respond to the crime and discover the girl has been murdered

How can we find male DNA?

- ◆ Rasp files can with cutting his or bed linens
- ◆ Did he cut himself
- ◆ Finding STR profile?
- ◆ Does it match aspects of female blood and only possible traces of male DNA
- ◆ probative?

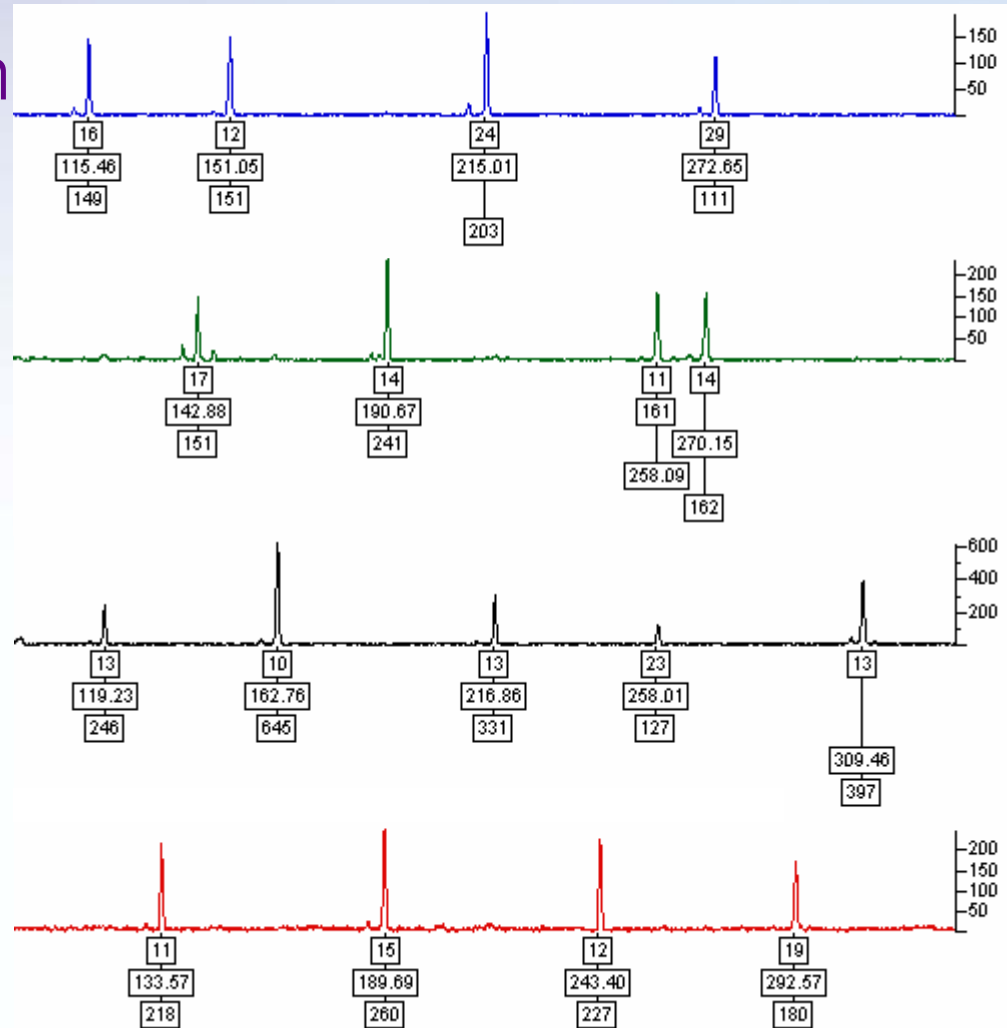


Case #3: Few/No Sperm are Detected

- ◆ Man is suspected of taking advantage of numerous young girls
- ◆ Provides them with alcohol
- ◆ Drugs them and takes them to his home
- ◆ Sexually assaults the girls
- ◆ Several girls are photographed
- ◆ Women shower
- ◆ Clothing is washed
- ◆ Thus far, no traces of male DNA
- ◆ Sample submitted: small stain from panties

Case #3, continued:

- ◆ Yfiler → full profile in epithelial fraction
- ◆ No results in sperm fraction
- ◆ EF profile matches suspect
- ◆ Database search:
 - ◆ 0 in 3561 males



Final Thoughts...



- ◆ If you can get “normal” DNA testing results, do it!
 - ❖ Y-testing should be reserved for cases when autosomal testing fails completely or is inconclusive
- ◆ All statistical power depends on how big the Y-profile database is – the bigger the better.
 - ❖ However, the likelihood of getting a unique profile will increase when testing more Y-STR loci
- ◆ Inheritance of the Y-chromosome is somewhat unique – all males from the same lineage have the same Y-profile.
- ◆ Y-chromosome testing can provide extremely valuable information in the genetic identification of males

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Contact Information

Cassie Johnson

Orchid Cellmark

13988 Diplomat Drive, Suite 100

Dallas, TX 75234

Telephone (direct): 214.271.8329

E-mail: cjohnson@orchid.com