

# **Implementation of the Quantifiler Duo DNA Quantification Kit as an Integrated Forensic Workflow Tool**

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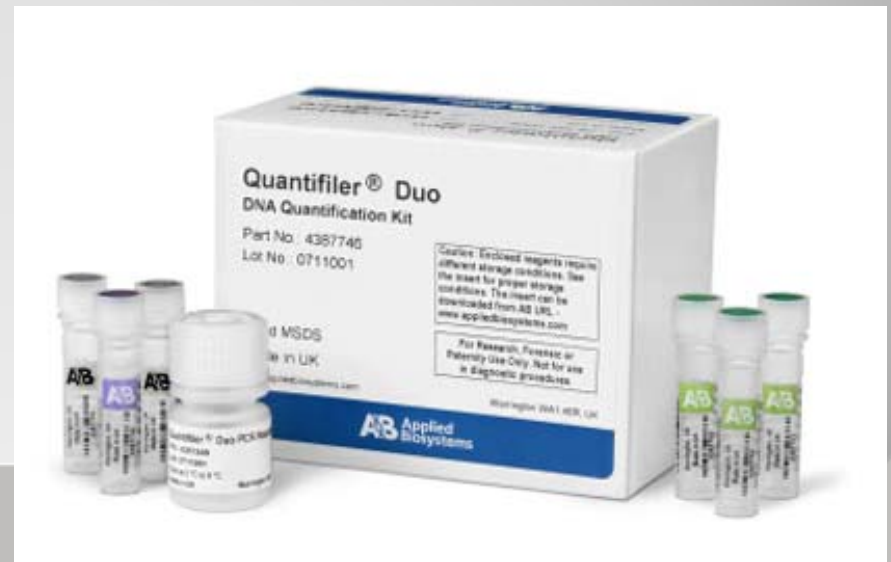
# OVERVIEW

- Introduction to Quantifiler Duo
- Internal Validation at SDPD
- Integration into Forensic Workflow
- Casework Experiences

**INTRODUCTION  
TO  
QUANTIFILER DUO**

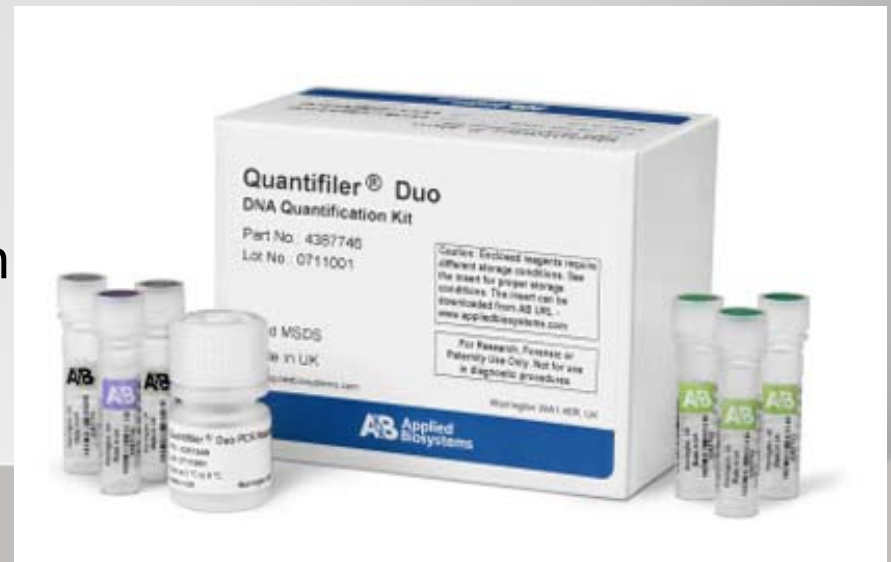
# QUANTIFILER DUO

- Multiplex DNA Quantification Kit
  - Total Human DNA
  - Total Male DNA
  - Internal PCR Control (IPC)
- TaqMan Technology
  - Same as Quantifiler Human/Y
- Single-Copy Targets
  - Correlates with STR kits
- Larger Amplicon Size
  - Quantification of STR amplifiable DNA
- Internal PCR Control
  - Identification of PCR inhibitors



# QUANTIFILER DUO

- Concentration Range
  - 0.023 ng/ $\mu$ L to >50 ng/ $\mu$ L
- Sensitivity
  - Down to 6 pg/ $\mu$ L
- Standard Curves from Pooled Human Male Genomic DNA
  - Improve reliability of concentration estimate
  - Better predict amplification success
- STR Kit Selection
  - Autosomal
  - Y-STR
  - miniSTR
- Optimized for 7500 Real-Time PCR System



# KIT COMPARISON

<u>KIT</u>	<u>TARGET</u>	<u>MARKER</u>	<u>LOCATION</u>	<u>Size</u>	<u>Dye</u>
Quantifiler Human	Human DNA	Human Telomerase Reverse Transcriptase (hTERT)	5p15.33	62 bp	FAM
	Internal PCR Control	Artificial Template	-	79 bp	VIC
Quantifiler Y	Human Male DNA	Sex Determining Region Y (SRY)	Yp11.3	64 bp	FAM
	Internal PCR Control	Artificial Template	-	79 bp	VIC
Quantifiler Duo	Human DNA	Ribonuclease P RNA Component H1 (RPPH1)	14q11.2	140 bp	VIC
	Human Male DNA	Sex Determining Region Y (SRY)	Yp11.3	130 bp	FAM
	Internal PCR Control	Artificial Template	-	130 bp	NED

**INTERNAL  
VALIDATION AT  
SDPD**

# SENSITIVITY STUDY

- Two samples amplified over a range of concentrations to determine optimum amount of input DNA for each kit
  - Tested ranges based on previous target amount

<u>KIT</u>	<u>TESTED RANGE</u>	<u>FULL PROFILE</u>	<u>ABI RECOMMENDED RANGE</u>	<u>SDPD OPTIMUM RANGE</u>
Identifiler	0.5-4.0ng	ALL	0.5-1.25ng	1.0ng
MiniFiler	0.1-1.0ng	ALL	0.5-0.75ng	0.3-0.4ng
Yfiler	0.1-2.0ng	ALL	0.5-1.0ng	0.5-0.75ng



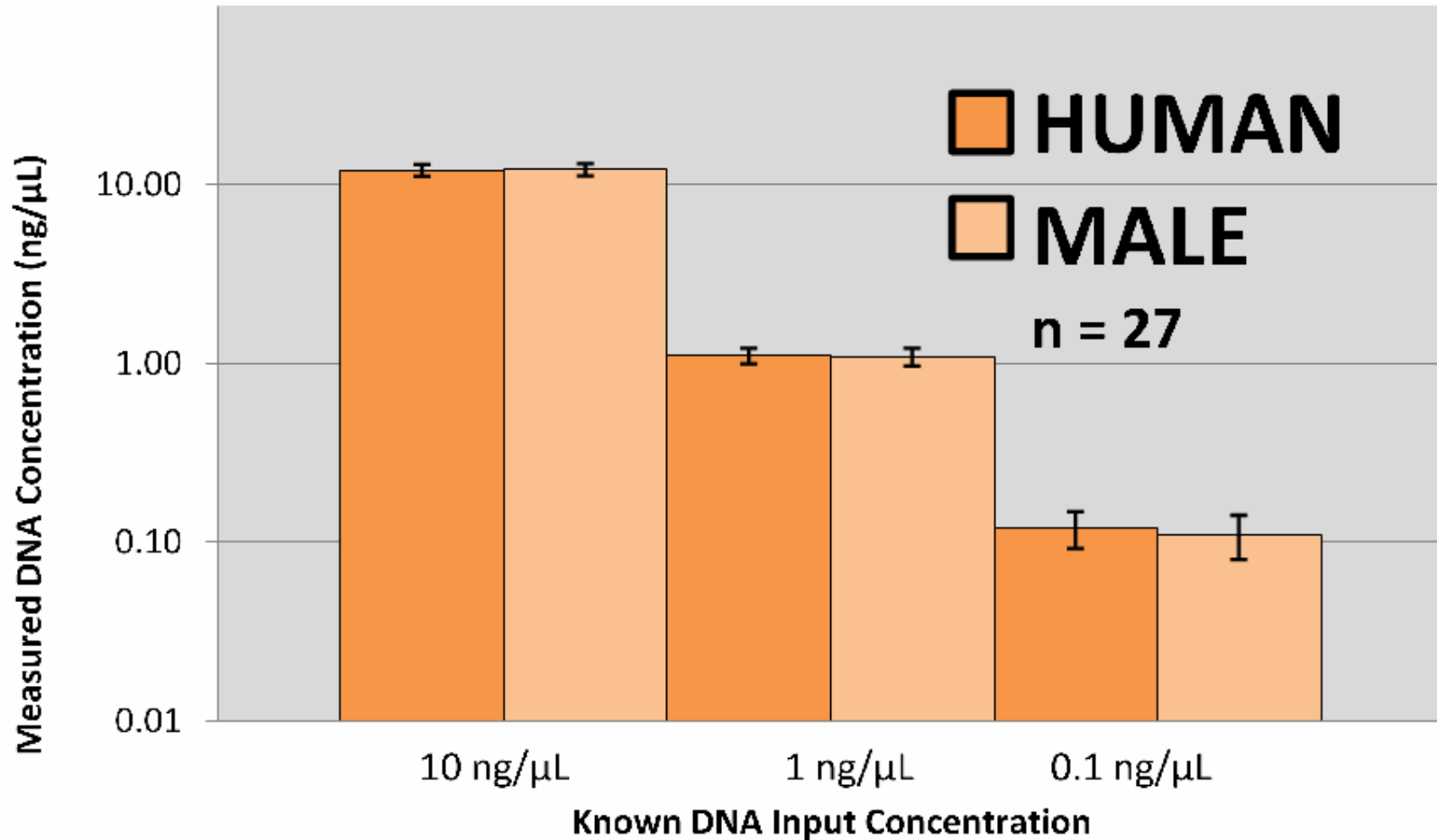
# MIXTURE STUDY

Male: Female	Male Profile Detected (Identifiler)	# of Male Loci Detected (Identifiler)
1:1	FULL	15
1:2	FULL	15
1:5	2 of 3 FULL	14-15
1:10	PARTIAL	10-14
1:20	PARTIAL	5-14
1:50	1 of 3 PARTIAL	0-5
1:100	NONE	0

# MIXTURE STUDY

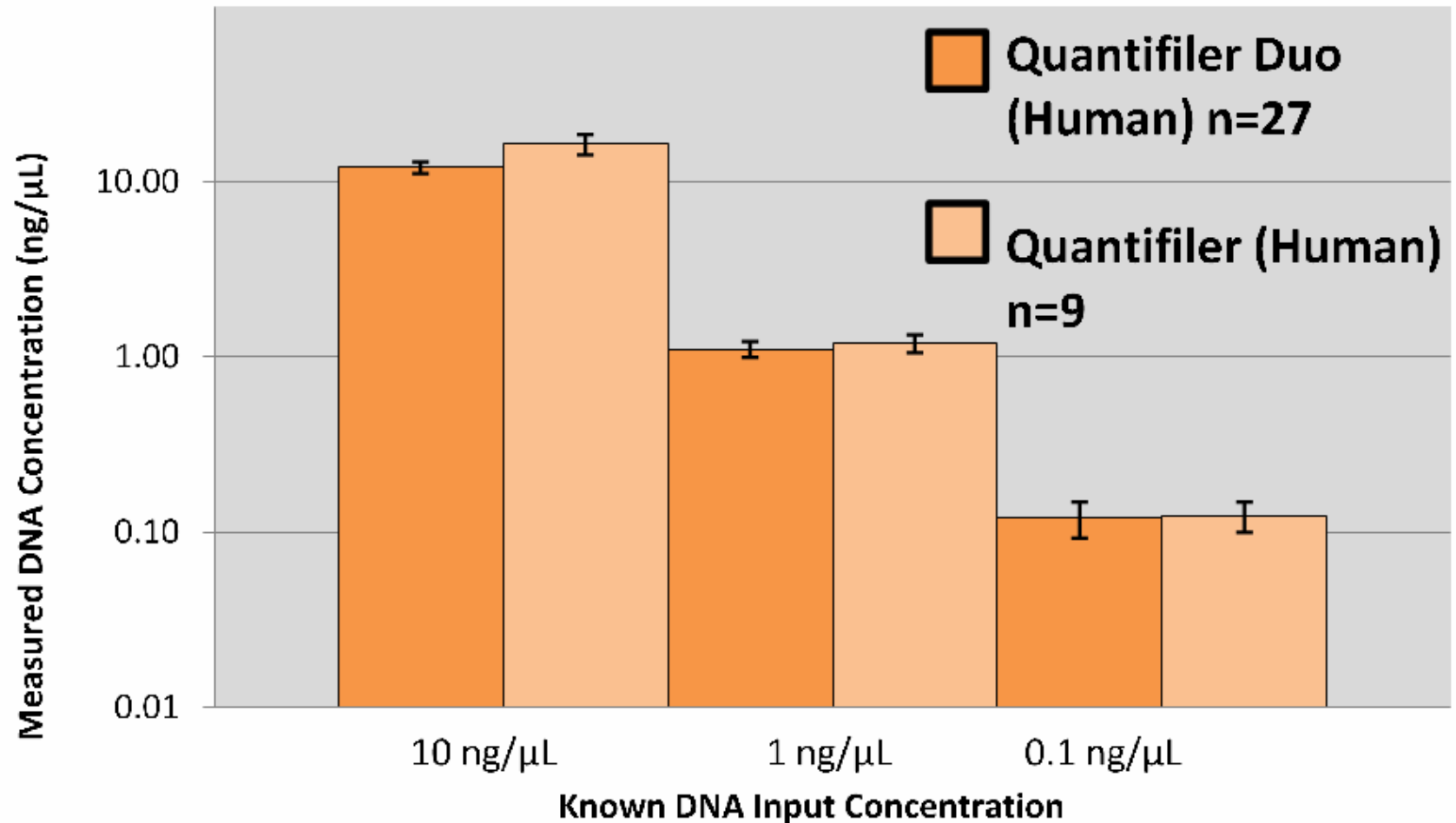
- If Ratio of Male:Female DNA is:
  - $\geq 1:5$  amplify using **IDENTIFILER**
  - **1:20 – 1:5** then consider both **IDENTIFILER** and **YFILER**
    - Yfiler haplotype may carry greater statistical weight than partial Identifiler profile (vice versa)
    - Other factors should be considered
  - $< 1:20$  amplify using **YFILER**

# REPRODUCIBILITY STUDY



Quantification results from replicate samples were similar within the same real time PCR plate and between plates

# QUANTIFILER HUMAN COMPARISON STUDY



- Quantifiler tends to overestimate amplifiable DNA

# ADJUDICATED CASES

- Five adjudicated sexual assaults

<u>Total Human: Male DNA</u>	<u>Identifiler Inclusion</u>	<u>Yfiler Inclusion</u>
<b>2000</b>	<b>ONLY VICTIM DNA</b>	<b>1 in 1391</b>
<b>46</b>	<b>MAINLY VICTIM DNA</b>	<b>1 in 1391</b>
<b>28</b>	<b>MAINLY VICTIM DNA</b>	<b>1 in 1391</b>
2.7	1 in 210 trillion	1 in 1391
2.6	1 in 1 billion	1 in 1391
1.8	1 in 72 quadrillion	1 in 1391
1.2	1 in 11 quintillion	1 in 1391
1.0	1 in 11 quintillion	1 in 1391
1.0	1 in 10 quintillion	1 in 1391
<b>NO MALE DNA QUANT</b>	<b>ONLY VICTIM DNA</b>	<b>1 in 1391</b>

•Ratio > 20: Yfiler is more probative than Identifiler

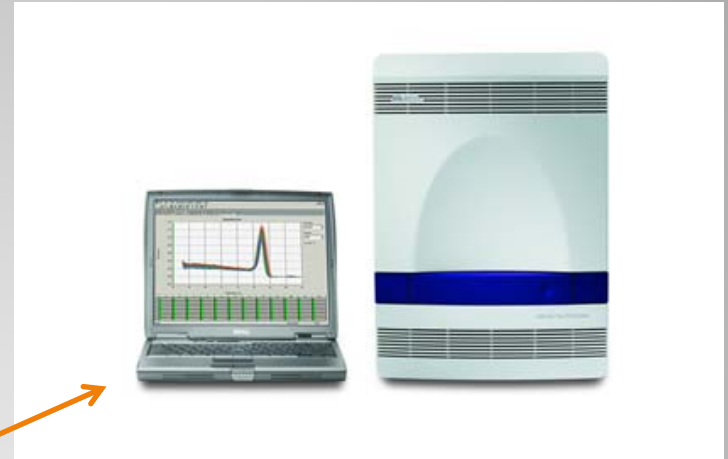
•Ratio < 6: Identifiler is more probative than Yfiler

•Partial Yfiler profile w/ negative male DNA result from Quantifiler Duo (80µL of extract concentrated prior to amplification)

**INTEGRATION  
INTO FORENSIC  
WORKFLOW**

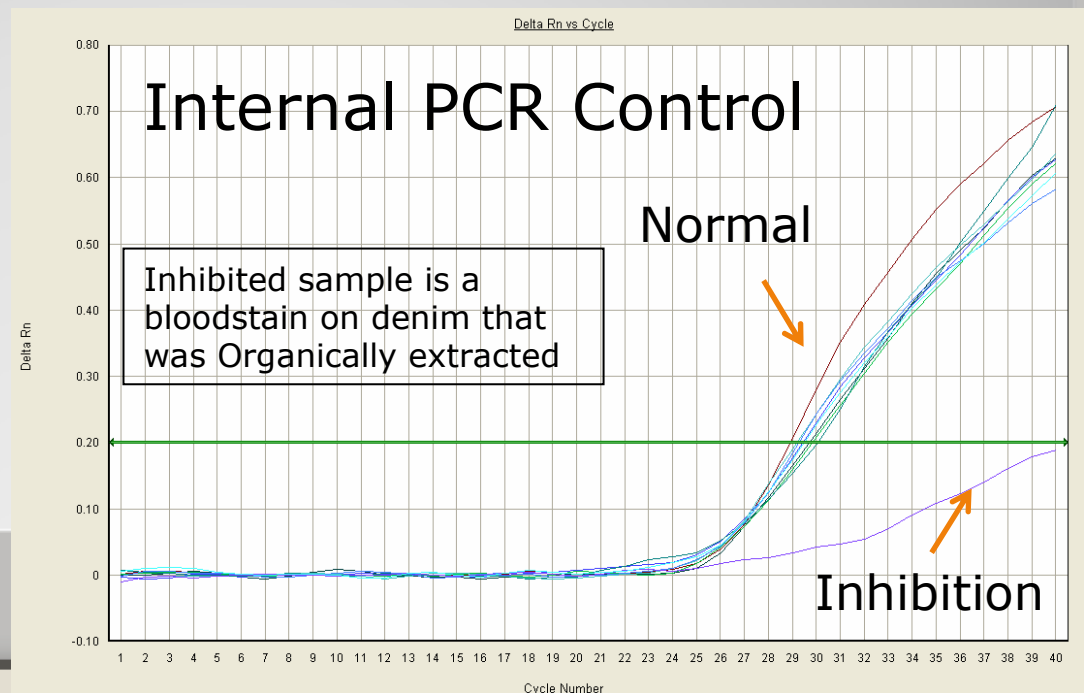
# QUANTIFILER DUO at SDPD

- Quantifiler Duo currently used for all SEXUAL ASSAULT casework (Quantifiler Human for all others)
  - Potentially all casework upon acquisition of a 2<sup>nd</sup> 7500 Real Time PCR System
- Manual and Automated quantification set-up protocols
  - Qiagen BioRobot Universal
  - Identical to Quantifiler set-up



# QUANTIFILER DUO at SDPD

- Information and Results Management
  - WORKSHEET MAKER
    - SDPD's Excel-based program
- Inhibition Assessment
  - Internal PCR Control
    - Dilute
    - Re-Purify
    - Additional Taq/BSA
    - MiniFiler
- STR Kit
  - Human: Male Ratio
    - Identifiler
    - Yfiler
    - MiniFiler





# WORKSHEET MAKER

SAN DIEGO POLICE DEPARTMENT  
FORENSIC SCIENCE SECTION  
Quantifiler Duo - QPCR Setup Worksheet

Case #: \_\_\_\_\_ Date: 6/27/2008 Analyst: \_\_\_\_\_

	1	2	3	4	5	6	7	8	9	10	11	12
A	50ng/uL Standard	50ng/uL Standard	9947A_Control	NS_1E	11-3	FS-2B	AT					
B	16.7ng/uL Standard	16.7ng/uL Standard	TE_Amp__Blank	NS_1F	FS6	FS-3A	KM					
C	5.56ng/uL Standard	5.56ng/uL Standard	1A	NS_1D	6A	FS-3B	EK					
D	1.85ng/uL Standard	1.85ng/uL Standard	1A	NS_1E	2	FS-3C	SK					
E	0.62ng/uL Standard	0.62ng/uL Standard	1A	NS_1A	3	4-1	JL					
F	0.21ng/uL Standard	0.21ng/uL Standard	RB	NS_RB	4	RB2	CA					
G	0.068ng/uL Standard	0.068ng/uL Standard	NS_1A	11-1	5	CF	CW					
H	0.023ng/uL Standard	0.023ng/uL Standard	NS_1A	11-2	FS-2A	AC	RBr					

Quantifiler Duo Kit Lot #: 0711001

ABI 7000/7500: 7500

Slope = -3.24

$r^2$  value = 0.997

Master Mix Prep:

Reagent	# of samples + 6	X vol (ul)	Total (ul)
QPCR Reaction Mix	62	12.5	775
QPCR Primer Sets	62	10.5	651

BioRobot Universal assisted setup

Manual setup

Standard dilution prepared by the BioRobot Universal

Standard serial dilution prepared manually

- Quantification Worksheets
- Automated Set-Up
  - .CSV file for Qiagen BioRobot Universal
- Sample Set-Up Import
  - .TXT file for 7500 System SDS Software
- Blank tab for import of Quantifiler Duo results

# WORKSHEET MAKER

WELL	SAMPLE NAME	QUANT VALUE (ng/uL)		H/M RATIO	KIT	INTERNAL PCR CONTROL				
		Human	Male			IPC CT	IPC CHECK	Inhibition	Taq/BSA or Dilute	MiniFiler
A3	Sample A	12.16	12.28	0.99	Identifiler	29.38	OK			
B3	Sample B	6.00	0.94	6.38	Both	29.49	OK			
C3	Sample C	0.13	0.08	1.58	Identifiler	31.56		Possible Inhibition	Taq/BSA or Dilute	
D3	Sample D	13.33	13.04	1.02	Identifiler	29.48	OK			
E3	Sample E	15.00	1.38	10.87	Both	29.53	OK			
F3	Sample F	10.00	0.12	81.97	Yfiler	29.49	OK			
G3	Sample G	9.82	0.07	142.32	Yfiler	29.44	OK			
H3	Sample H	1.07	1.13	0.95	Identifiler	29.30	OK			
A4	Sample I	0.17	0.12	1.39	Identifiler	36.89		Possible Inhibition		MiniFiler?
B4	RB	0.00	0.00	-	Identifiler	29.53	OK			

- Quantifiler Duo Results Interpretation
- Master Amplification Worksheet/ Qiagen BioRobot Universal Identifiler Set-Up file
- Identifiler Amplification Worksheet
  - Yfiler and MiniFiler Amplification Worksheets created manually

# **CASEWORK EXPERIENCES**

# CASEWORK EXPERIENCES

- Prior to Quantifiler Duo, SDPD had no quant method for male DNA
- Quantifiler Duo approved for casework June 18, 2008
- Seven batches of cases
  - Evidence and reference samples from 32 different cases
  - Mainly sexual assaults w/ few others

# CASE #1

- October 15, 2006 (2:30 am)
- Two male and two female college students inside Mission Beach condominium
- Three males near Belmont Park looking to commit robberies



# CASE #1 (cont.)

- The three males enter the unlocked condo
- Armed w/ handguns
- Gang rape/sodomize female victims; force male victim to have sex w/ female
- Steal television, credit cards, cell phones...
- Use stolen credit cards and cell phones
  - Traced to three suspects:
    - Willie Watkins
    - Donald Smith
    - Antonio Washington

# CASE #1 (cont.)

- DNA Testing at SDPD
  - Autosomal STRs
    - Donald Smith and Antonio Washington linked to crime (no solid DNA evidence of sex component)
    - Most samples gave results consistent with victims
  - Y STRs
    - No Y quant method at SDPD
    - Identifiler attempted 1<sup>st</sup>
      - Consumed much of DNA extract
    - Resulted in no profile or partial profile for most samples

# CASE #1 (cont.)

- DNA Testing at SERI (private lab)
  - Y STRs
    - Human and Y quant available
    - DNA from Willie Watkins, Donald Smith, and Antonio Washington on vaginal/external genital swabs from both female victims
    - Linked suspects to sex component of crime not just robbery
- Private lab obtained better Y-STR results than SDPD due to availability of Y quant
- Quantifiler Duo would have been a valuable tool!!!



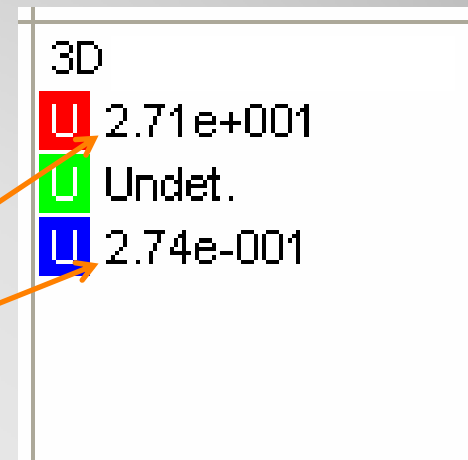
# CASE #1 (cont.)

- Willie Watkins, Donald Smith, and Antonio Washington convicted of 76 felony counts including forcible rape, robbery, and kidnapping
- Each sentenced to more than 316 years to life (maximum under CA law)



# CASE #2

- Sexual assault victim alleges suspect orally copulated her
- Cutting from victim's panties analyzed
  - Amylase positive
- Quantifiler Duo results:
  - Total Human = 27.1 ng/ $\mu$ L
  - Total Male = 0.274 ng/ $\mu$ L
- Nearly 100 times more female DNA present than male DNA

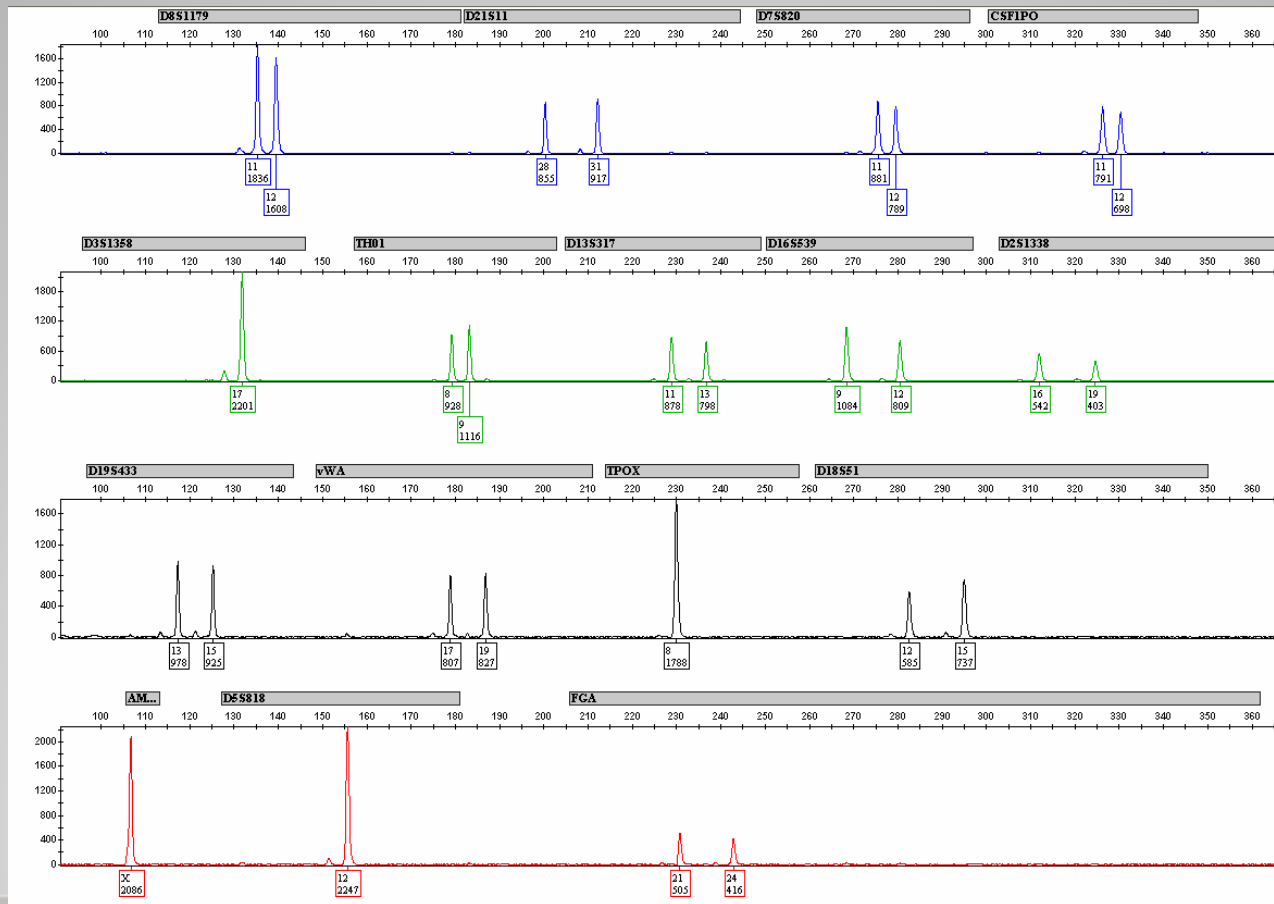


A table showing Quantifiler Duo results. The table has three rows of data. The first row shows a red 'U' icon and the value 2.71e+001. The second row shows a green 'U' icon and the value Undet. The third row shows a blue 'U' icon and the value 2.74e-001. Two orange arrows point from the text 'Total Human = 27.1 ng/μL' in the main text to the red 'U' icon and the value 2.71e+001. Another orange arrow points from the text 'Total Male = 0.274 ng/μL' to the blue 'U' icon and the value 2.74e-001.

3D	Value
U	2.71e+001
U	Undet.
U	2.74e-001

# CASE #2 (cont.)

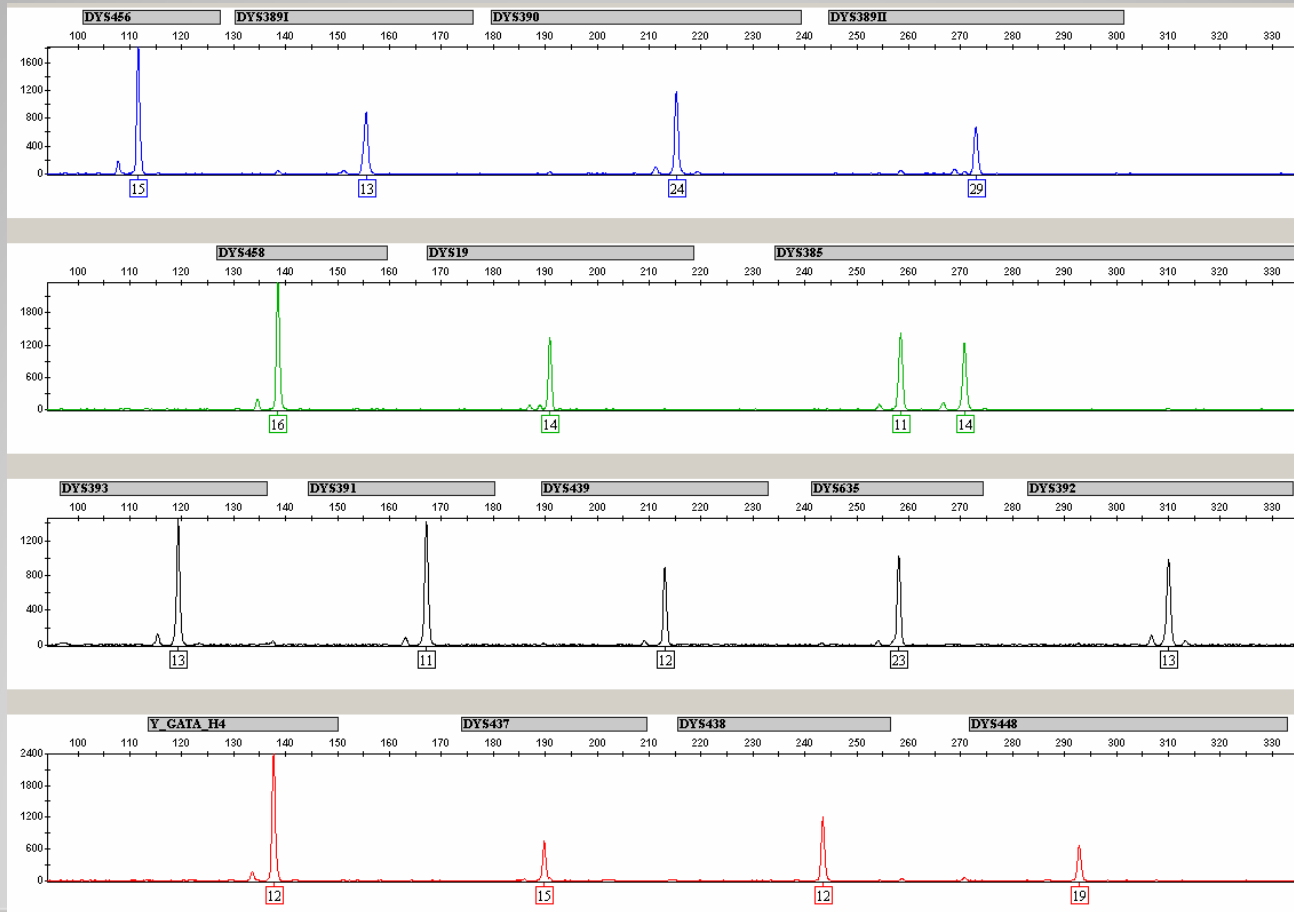
- Identifiler Results



- Single source female profile consistent w/ victim

# CASE #2 (cont.)

- Y-File Results



- Single source male haplotype consistent w/ suspect

# CASE #2 (cont.)

- Y-Filer Results
  - Statistical Significance:
    - Number of times observed in US Y-STR Database
      - 1 in 4165 tested individuals
  - Without Quantifiler Duo wouldn't have had information about the low level male contributor!!!

# CASE #3

- Victim at bar offered ride home
  - Raped in van
- Quantifiler Duo results:
  - Right breast swab
    - Total Human = 0.0866 ng/ $\mu$ L
    - Total Male = 0.0226 ng/ $\mu$ L
      - Male:Female DNA Ratio = 1:2.8
    - Concentrated 30  $\mu$ L to 8  $\mu$ L
      - 2.61 ng total DNA (normal target 1 ng)
      - 0.678 ng of male DNA amplified
- DNA Typing results:
  - Mixture of victim and foreign male
  - No apparent drop-out of foreign alleles
  - Entered into CODIS

U	8.66e-002
U	Undet.
U	2.26e-002

# CASE #4

- Victim (V) kidnapped, raped, and assaulted over two day period
  - V was made to shower twice
  - Deep vaginal swab submitted for DNA testing
- Quantifiler Duo results (sperm fraction):
  - Total Human = 0.105 ng/ $\mu$ L
  - Total Male = 0.039 ng/ $\mu$ L
- Concentrated 30 $\mu$ L to 8 $\mu$ L
  - 1.17ng of male DNA
  - 1.83ng of female DNA
- Both the V and S included
  - Likelihood Ratio:

Population	Statistics
Caucasian	1 in 260 quadrillion
African American	1 in 4 quintillion
Hispanic	1 in 400 quadrillion

# CASE #5

- Burglar enters victim's (V) residence
- V asleep in bed raped at knife point
- V hit suspect (S) in head with glass
- V cut with knife and stabbed w/ glass shard
- V is smoker; S smokes two of V's cigs
- S forces V to shower, washes bedding, bleaches floor
- 17 cig butts collected from V's bedroom
- Multiple bloodstains collected



# CASE #5 (cont.)

- 17 cig butts extracted and quantitated with Quantifiler Duo
  - 15 yield only female DNA
  - 2 yield male DNA (had been in glass of soda)
- Only samples with male DNA analyzed
  - Local CODIS database hit to 2006 burglary suspect
  - 17 yr old S arrested w/ wounds on head
  - 1<sup>st</sup> arrest at 8 yrs old for assault
  - Previously linked by DNA to 2 burglaries and 1 sexual assault at SDPD
  - Released from juvenile boot camp days prior

# CASE #5 (cont.)

- Quantifiler Duo as screening tool
  - 2 of 17 cigs w/ male DNA
  - 15 female DNA samples not amp/analyzed
    - Analyst time and resources saved
- Helped rapidly identify S
  - S wounds consistent w/ V's story
  - S planned to leave the state
- Quantifiler Duo will be used to screen the blood samples for male DNA

# SUMMARY

- Quantifiler Duo
  - Total Human and Total Male DNA quantitation in single reaction
  - Additional improvements over Quantifiler Human/Y
- Validated at SDPD for casework
- Proven to be a valuable tool in actual casework!!!

# ACKNOWLEDGEMENTS

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