



Enhancing the Sexual Assault Workflow: Testing of Next Generation Assessment and Y-STR Systems



J. Ballantyne^{1,2}, E. Hanson¹, R. Green³, A. Holt³ and J. Mulero³

¹National Center for Forensic Science, University of Central Florida, Orlando, FL USA

²Department of Chemistry, University of Central Florida, Orlando, FL USA

³Human Identification, Life Technologies, Foster City, CA

INTRODUCTION

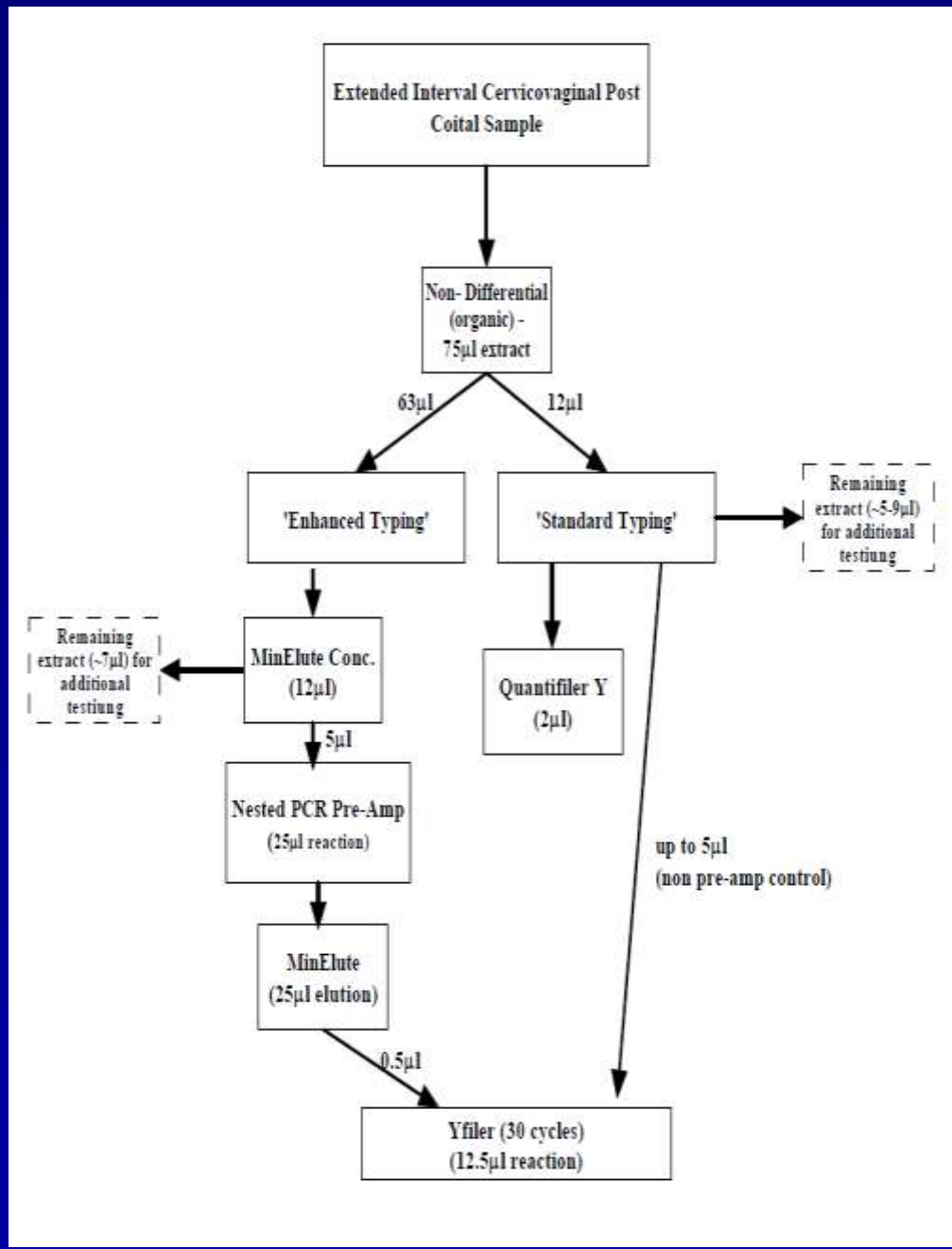
Sexual assault samples are among the most difficult samples types encountered by forensic laboratories. Typically a sexual assault sample has multiplex challenges including small quantity of male DNA, relatively high quantity of female DNA and the presence of PCR inhibitors. Therefore, there is a need for a more robust, highly sensitive and faster method for assessment (i.e. quality and quantity) of DNA extracts to determine optimal downstream processing methods, as well as an improved Y-STR amplification system for profiling these difficult samples.

We tested newly developed tools, including a DNA quantification kit (Quantifiler® Trio) that exhibits high sensitivity, higher inhibitor tolerance and includes additional tools useful for the determination of DNA quality to inform downstream processing methodology. We also tested a new Y-STR kit (Yfiler® Plus) with enhanced capabilities. This 27-marker kit permits rapid amplification of single source and complex casework samples.

These two next generation systems are intended to generate an improved workflow for obtaining interpretable profiles from sexual assault samples. Here, we present the utility of the new workflow in processing sexual assault samples. We have successfully used the Quantifiler® Trio quantitation and assessment kit and the Yfiler® Plus amplification kit to obtain informative Y-STR profiles from challenging sexual assault sample types, including extended interval post coital samples collected 4, 7 and 9 days after intercourse (28 sets), which contained extremely low quantities of male DNA and high amounts of female DNA (1:333 male:female DNA ratios or greater). Probative Y-STR profiles were obtained from these samples using the next generation quantification and amplification systems with mostly full profiles obtained if >100pg of male DNA was present. Additionally, a good correlation between male quantitation and profile recovery was observed, with a negative quantitation value indicating that a negative or unusable profile would be obtained.

Since the examination of 4-9 day post coital samples is not routinely performed by operational crime laboratories, we also performed an analysis of one set of 1, 2 & 3 day samples. We report the use of Quantifiler® Trio, GlobalFiler™ and Yfiler® Plus to obtain full autosomal and Y-STR profiles.

MATERIALS AND METHODS



SAMPLES: Collaborative Study with University of Tennessee

- 69 kits with reportable results (74 received)
- 4, 7 and 9 day samples (collected after separate acts of intercourse)
- Cervix and fornix samples
- Original study designed for comparison of 'standard' analysis: Yfiler® and 'enhanced analysis' (Y-nested pre-amplification)
- Only one baseline per kit (i.e. not before every sample) used
- Donors allowed to have other sexual contact during collection intervals ("sex with condoms"; number of days in which this occurred during sample collection was recorded)
- Original experimental schema shown (left): manual organic extraction (non-differential)
 - Results clearly demonstrated improved profile recovery using Y-specific nested PCR pre-amplification ("enhanced")
- Next generation YSTR kit PowerPlex® Y23 available
 - Considered alternative "enhanced" method
- Results from a limited number of sample testing demonstrate the ability to use NGY kits to possibly improve profile recovery
- Current study: further evaluation NGY kits: Quantifiler® Trio and Yfiler® Plus

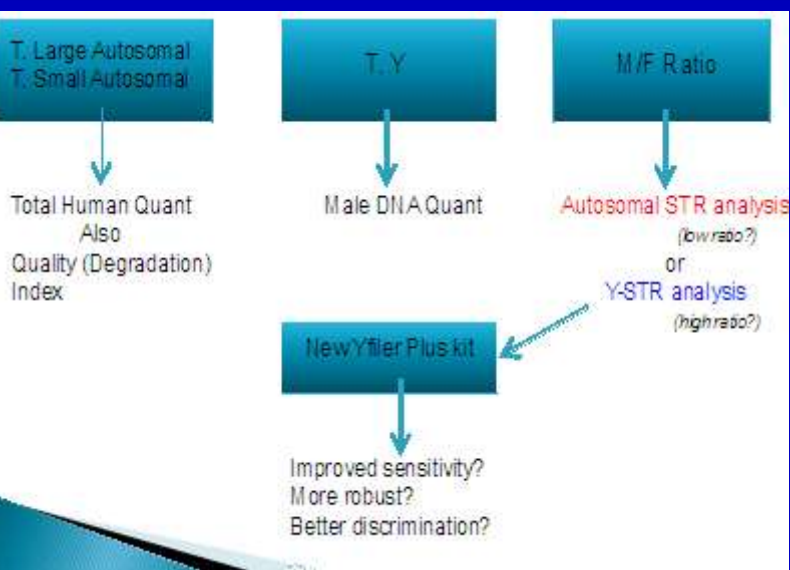
Quantifiler® Trio: manufacturer's protocol followed

- Performed on a 7500 Real Time PCR Instrument
- Data analysis performed using HID Real-Time PCR Analysis Software v1.1

Yfiler® Plus: manufacturer's protocol followed

- All amplifications performed using a 9700 thermal cycler
- Product detection was performed using a 3130 Genetic Analyzer (POP-7; GeneScan™ 600 LIZ® Size Standard)
- Profiles were analyzed using GeneMapper IDX v1.4

QUANTIFILER TRIO



Fast, accurate decision tool

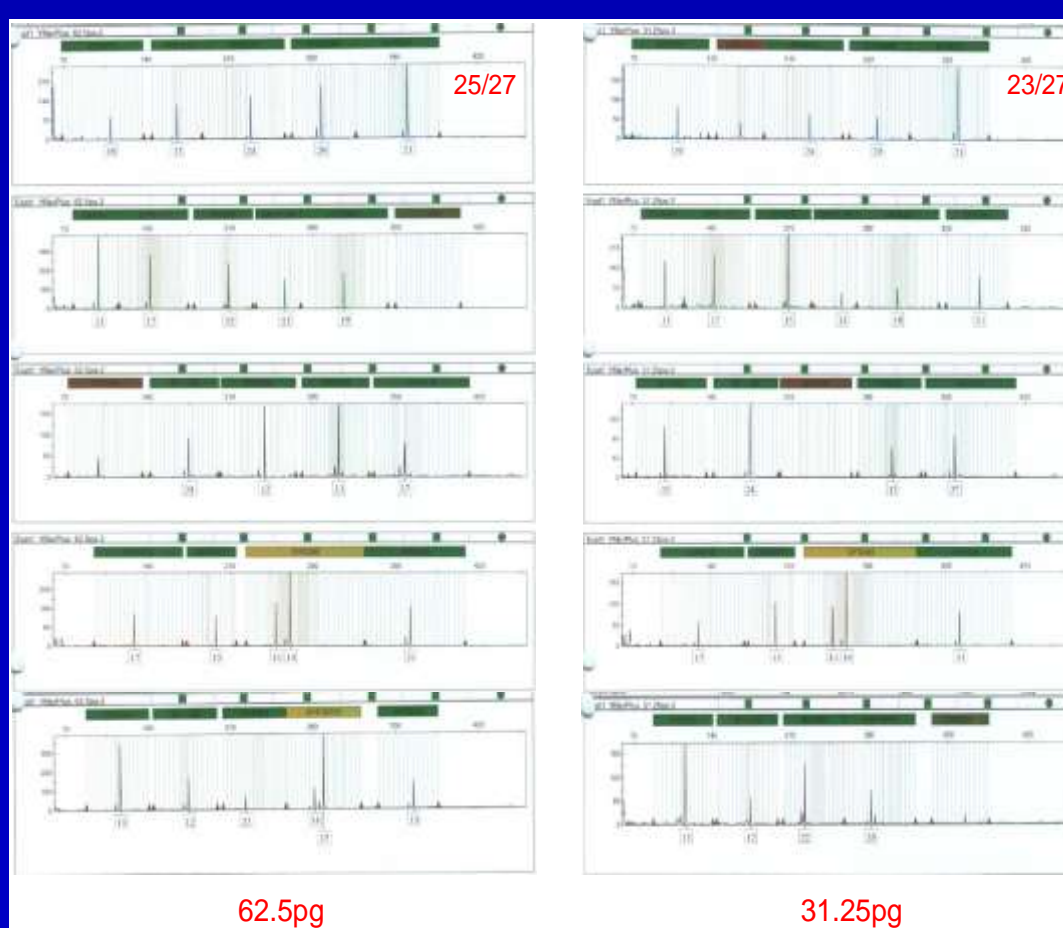
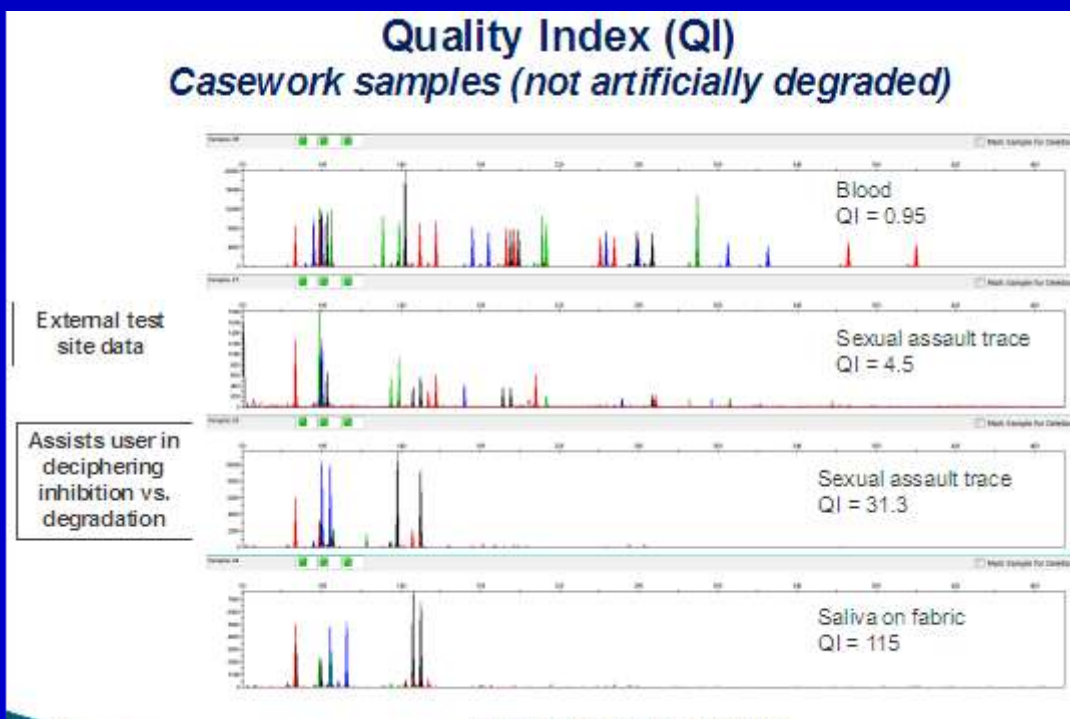
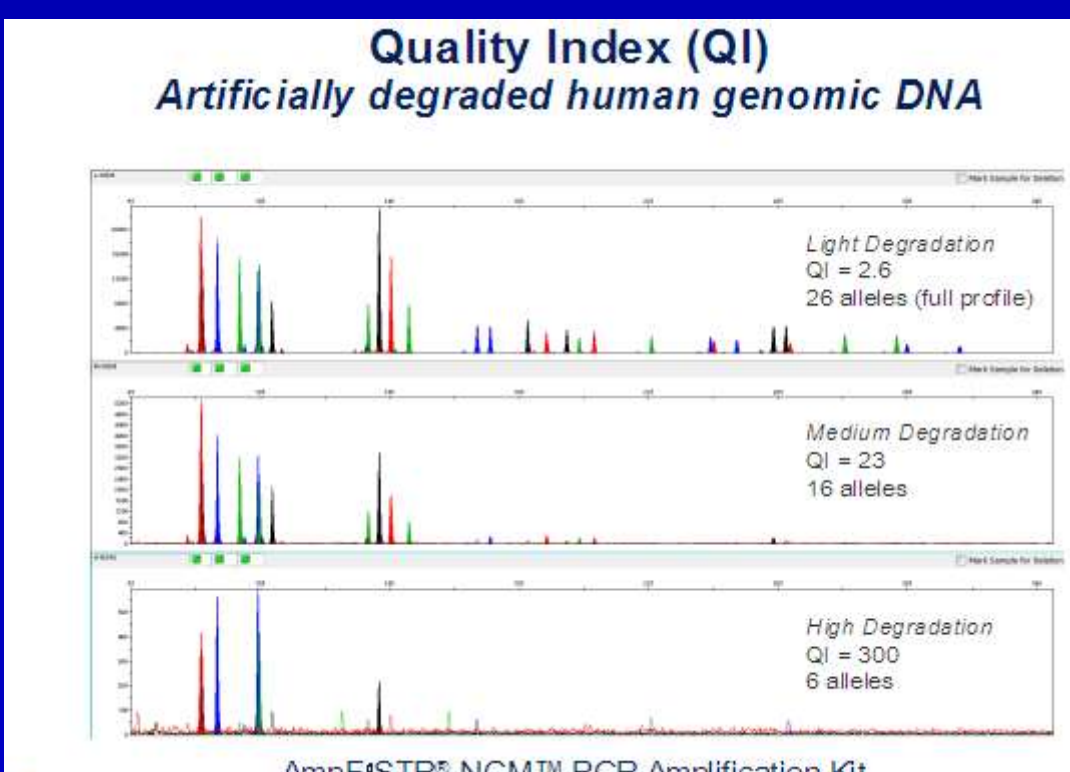
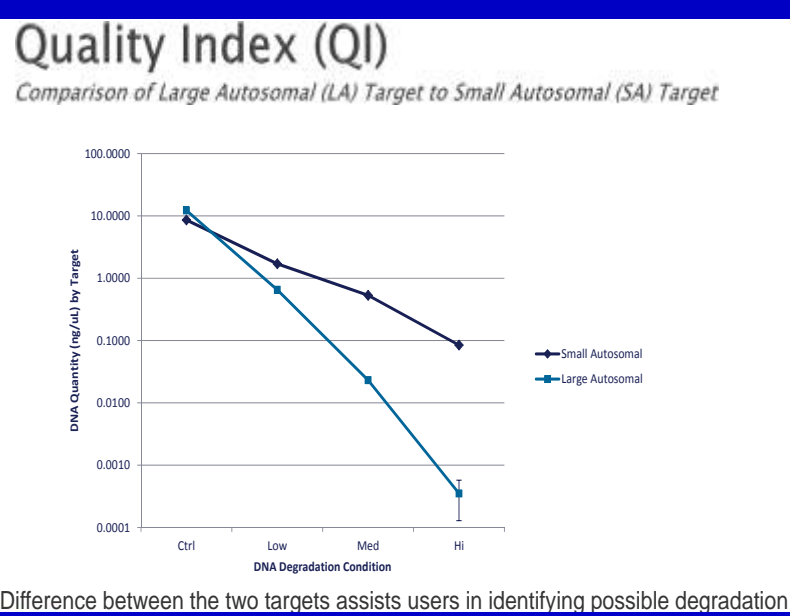
- Short analysis time (<1 hour)
- Increased assay sensitivity and accuracy
- Decision making for forensic casework sampling processing pathways?

Higher performance with challenging samples

- Improved performance with low-ratio M:F mixtures
- IPC optimization to match performance of NG STR kits
- Gain information on sample quality prior to PCR amplification (Quality Index, QI)

Enhanced workflow and data analysis

- Flexible protocols for improved sample throughput
- Improved consistency and homogeneity of DNA standard
- Customized workflow templates plus data analysis and STR setup tools



YfilerPlus	Quantifiler	Amplification	Total
Number of Individuals	100	100	200
Observed haplotypes	100	100	200
Discriminatory Capacity	100%	100%	100%
Occurrence of Most Frequent haplotype (%)	1 (1%)	1 (1%)	1 (0.5%)

Yfiler	Quantifiler	Amplification	Total
Number of Individuals	100	100	200
Observed haplotypes	95	100	195
Discriminatory Capacity	95%	100%	98%
Occurrence of Most Frequent haplotype (%)	2 (2%)	1 (1%)	2 (1%)

YFILER PLUS

Plus in Power of Discrimination

- Includes all 17 loci in Yfiler® plus 10 new Y-STR loci including 7 rapidly mutating Y-STRs
- Additional discrimination observed in 200 sample population study (left, bottom)

Plus in Performance

- Improved results with challenging case work samples
- Direct amplification protocol with single source samples
- Shorter Amplification times

Plus in Data Analysis

- Expanded allelic ladder

VTS Workbook summary

Sensitivity

- Significant partial profiles with as little as 31.25 – 62.5pg male DNA (left, top)
- Avg. No. of Alleles = 17 and 25 alleles, respectively

Inhibitors

- Full profiles in presence of high concentration of hematin (20, 200 µM) and humic acid (10, 100 ng/µl)
- 'Yfiler®' inhibited (no male profiles) at higher conc.

Mixtures

- Full male profiles in 1:1000 and 1:4000 male/female mixtures
- Minor male profiles detected in 1:8 male:male mixtures

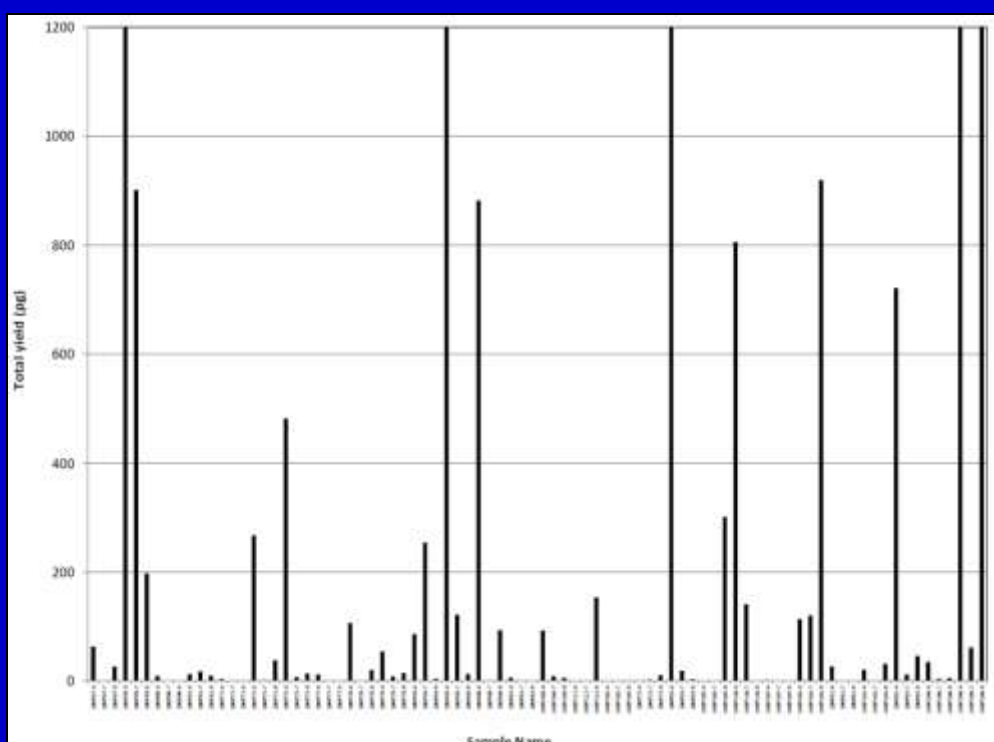
EXTENDED INTERVAL POST COITAL SAMPLES (4-9 DAYS)

Goals :

- Quantifiler® Trio
 - Improve quantitation sensitivity?
 - Majority of standard extracts had "undet" Quantifiler® Y values in previous testing
 - M/F ratio determination
 - How much female DNA are we really dealing with?
 - Does "undet" quant result truly correlate with no profile recovery?
- Yfiler® Plus
 - Improve profile recovery (i.e. sensitivity)
 - Eliminate need for pre-amplification?
 - Ability to recover profiles with vast excess of female DNA?

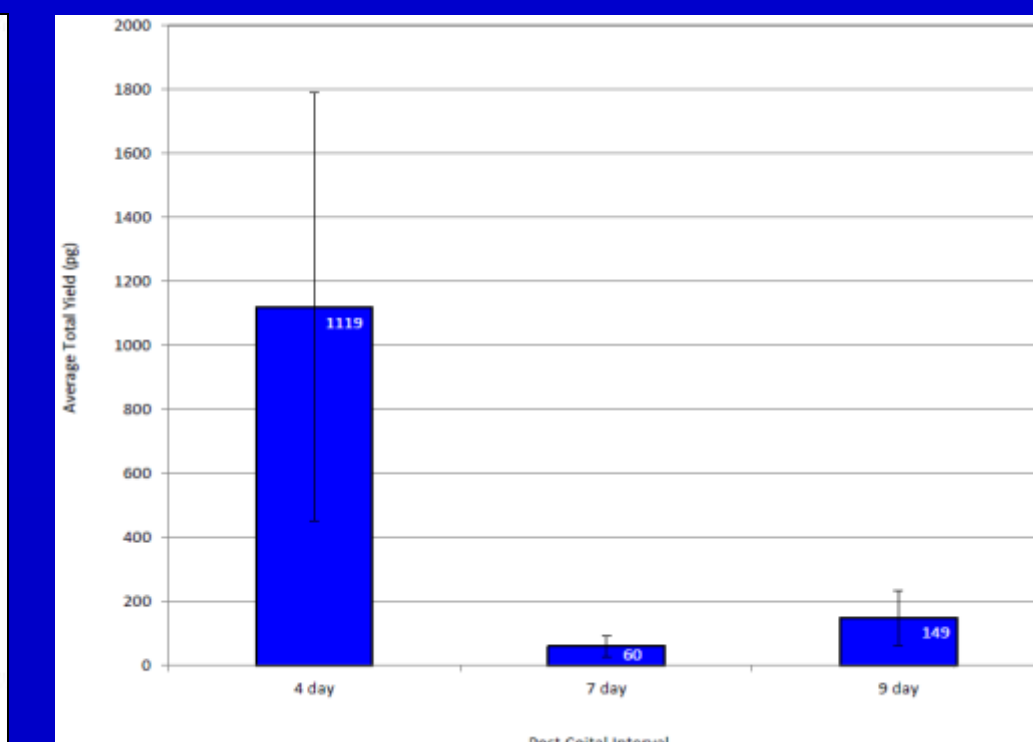
Samples: 28 of 69 UTHSC kits (~40%)

Total Yield – Male DNA (ng)



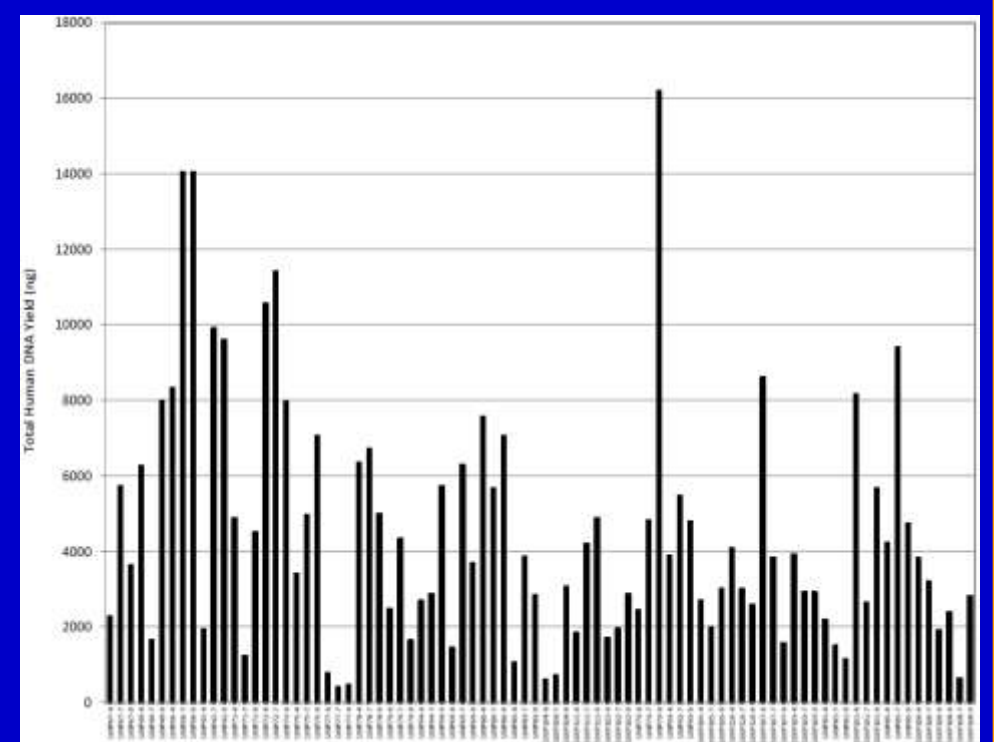
- ~15 µl extracts; N= 28 kits (84 samples)
 - Closer look at low range
 - A few samples with higher quants
 - 5 samples > 1ng (1, 2, 2, 6, 18 ng)

Average Total Male DNA Yield (pg)

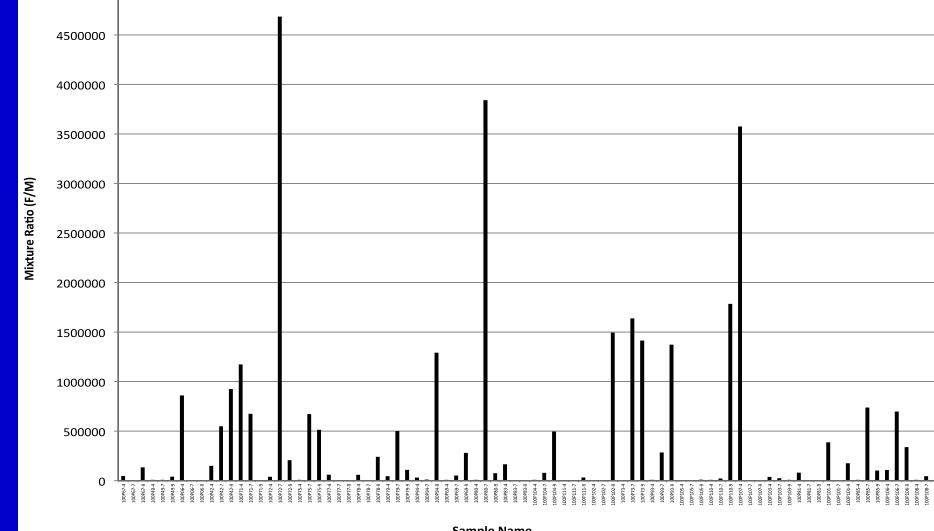


- Averages by time interval
- Greater male DNA recovery in 4 day samples (as expected)
- Large variation

Total Yield – Human DNA (ng)



- Based on T. Small Autosomal Values
- ~0.5 – 16 µg
- Most > 2 µg

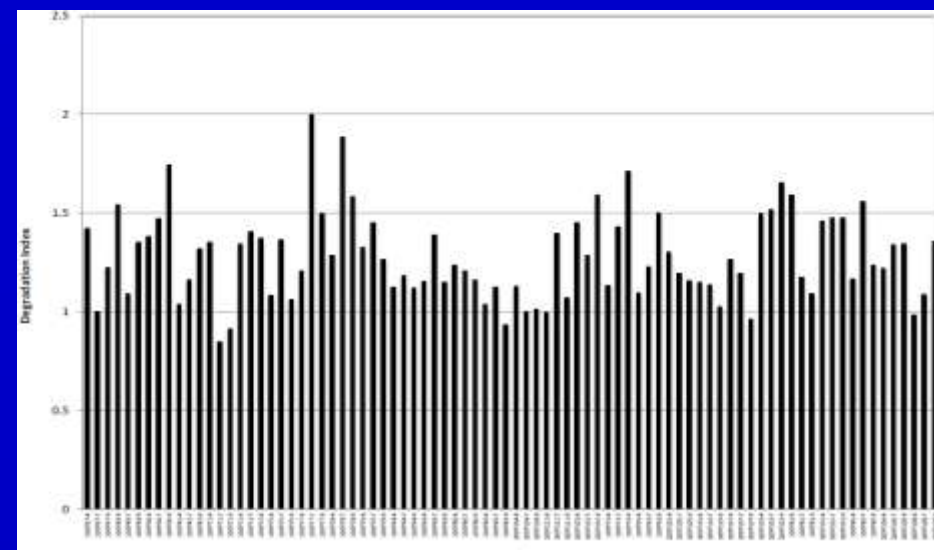


F:M Ratio

- ~333:1 to 4,685,117:1
- AutoSTR not possible

Quality (Degradation) Index

- No evidence of significant degradation
- Is sperm DNA quality masked?

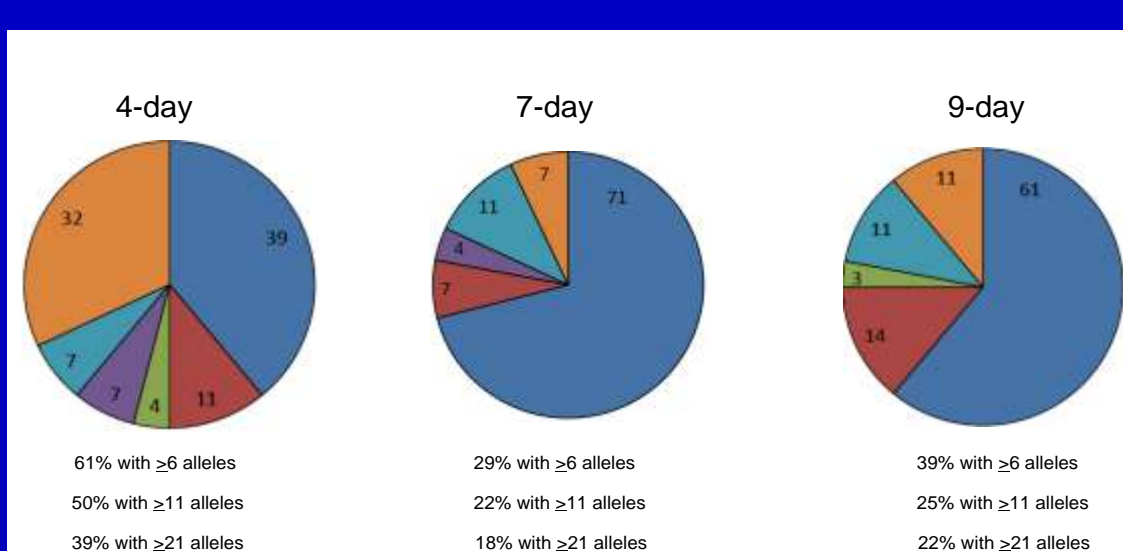
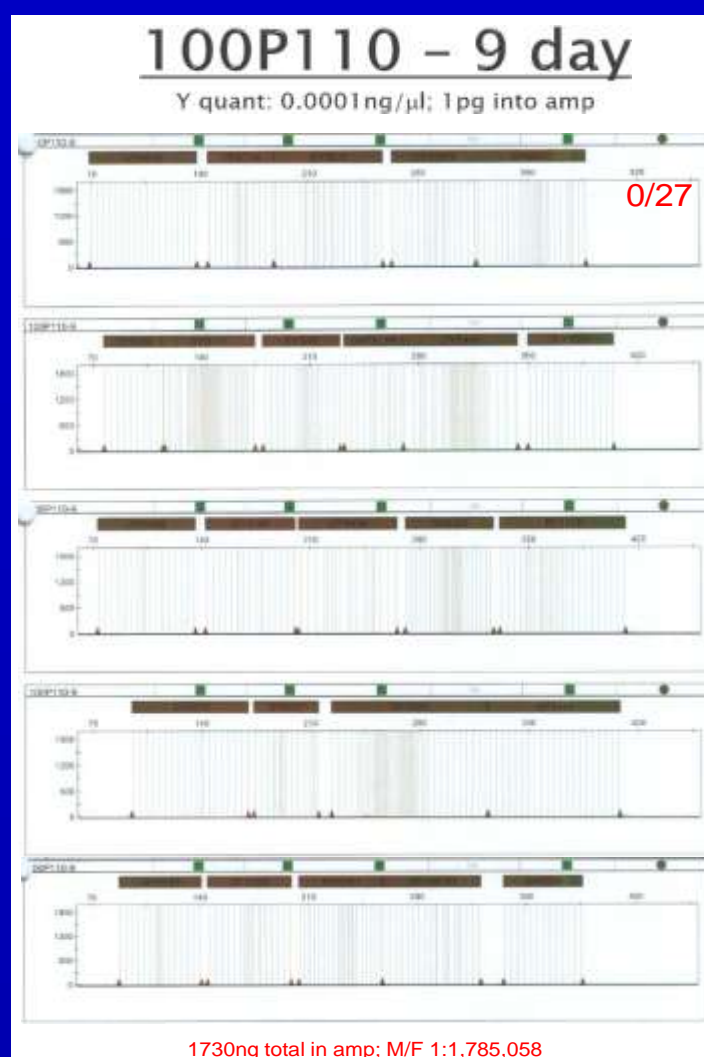
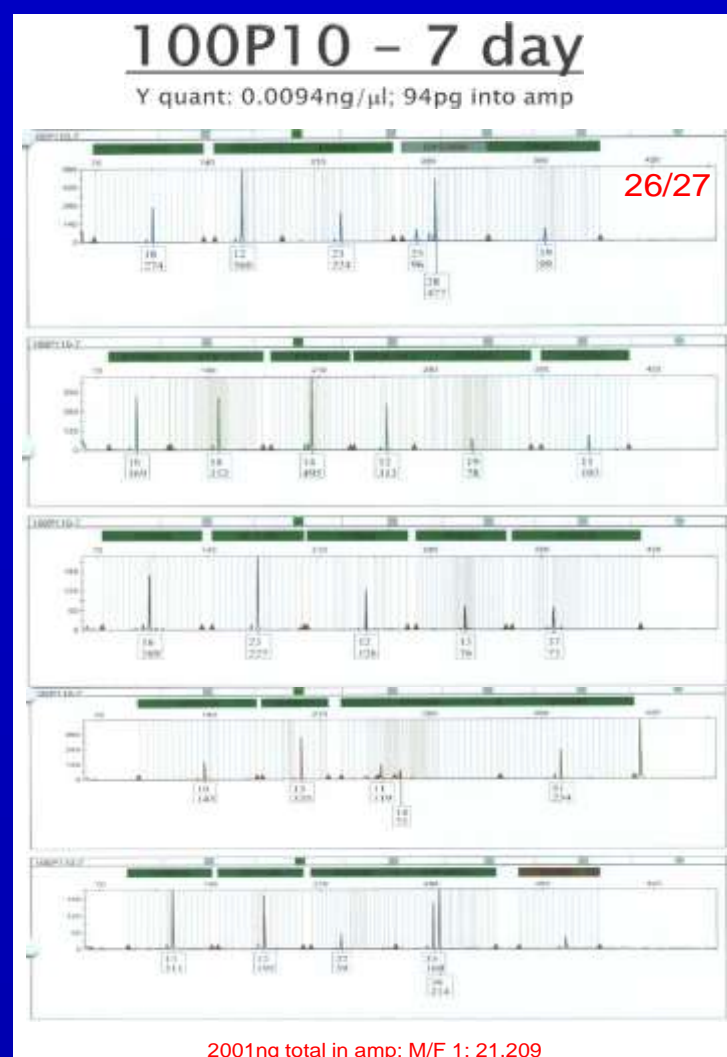
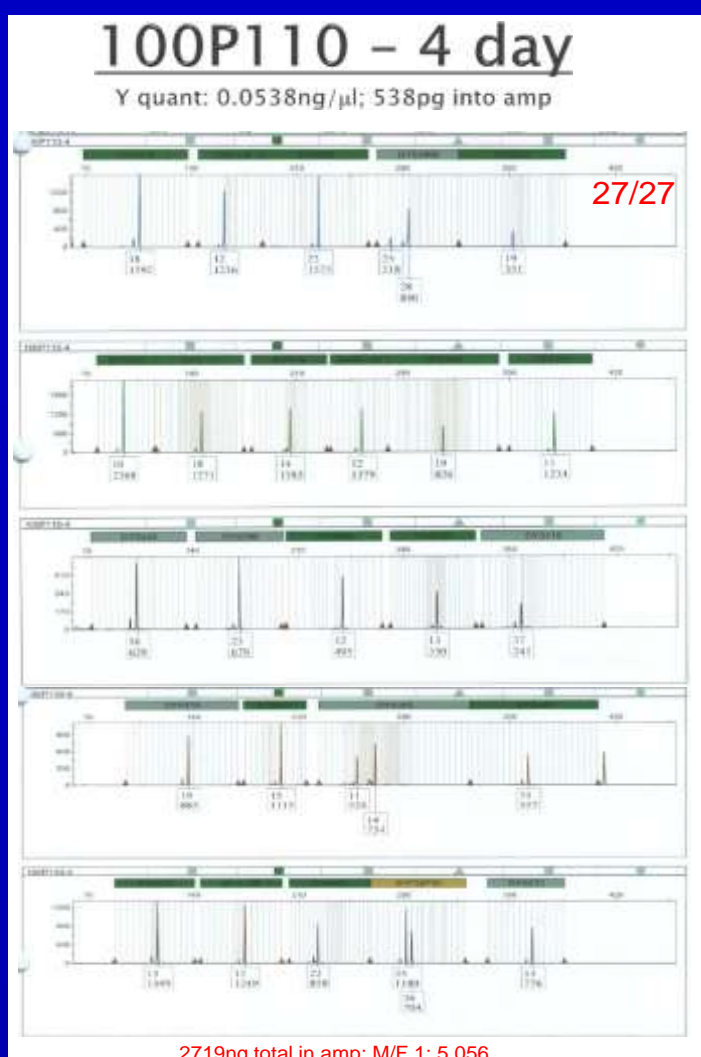


Input Male (ng) Yfiler® Plus

- Wide range of input
- Up to 1ng

Input Human DNA (ng) – Yfiler® Plus

- Majority with > 1µg



Yfiler® Plus Profiling Success Rate

- Percentage of full profiles for each interval
- Significant % of probative profiles (≥16 alleles)
 - 4 day – 46%
 - 7 day – 22%
 - 9 day – 22%

No. of Alleles vs. Total Male DNA (pg) in Amp (Low range shown)

- More alleles as input increases
- More or less complete profiles (>24 loci) with 100pg male DNA

1-3 DAY POST COITAL SAMPLES: AUSOTOMSAL AND YSTR PROFILING

Decision Making - Expected Results?									
Post coital Sample	Fraction	T. Large Autosomal (ng)	T. Small Autosomal (ng)	Total Human (ng in 50µl)	T. Y (ng)	Total Male (ng in 50µl)	M:F Ratio	Observed Results	Global Filer
1 day	NSP	269	262	14600	0.5117	26	1.569	1.087	Female profile only expected
	SP	3.1871	2.2535	113	2.1256	106	0.708	0.857	Male profile expected
2 day	NSP	33	28	1400	0.0027	0.14	1.10249	0.857	Female profile only expected
	SP	0.615	0.4144	21	0.2468	12	0.674	0.857	Male profile expected
3 day	NSP	187	185	9250	0.0037	0	0.807	0.857	Female profile only expected
	SP	0.0037	0.0029	0				0.807	No profile expected

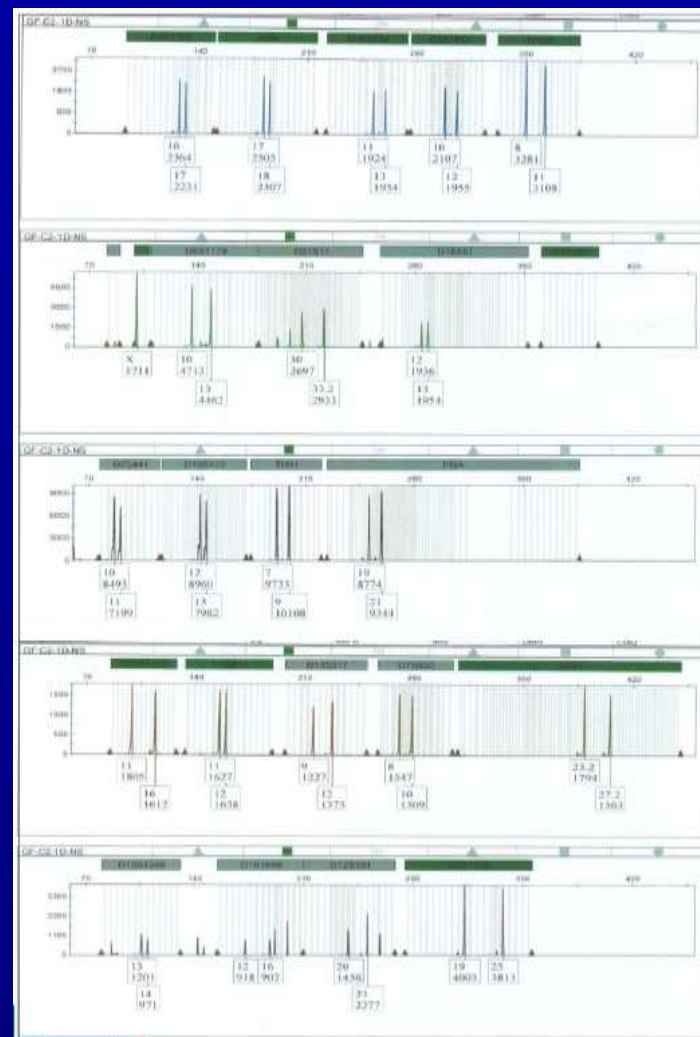
- In addition to the extended interval samples (4-9 days), we also wanted to evaluate more routinely encountered intervals: 1, 2 and 3 days
- Differential extraction (manual organic)
- Samples quantitated with Quantifiler® Trio
 - No degradation
 - Decision making: GlobalFiler™ (autosomal STRs) or Yfiler® Plus (Y-STRs) (*top panel*)
 - All samples amplified with both kits to determine if "predictions" or "decisions" were good (*bottom panel*)
- Observed results consistent with information gained from Quantifiler® Trio

Global Filer									
Post coital Sample	Fraction	T. Large Autosomal (ng)	T. Small Autosomal (ng)	Total Human (ng in 50µl)	T. Y (ng)	Total Male (ng in 50µl)	M:F Ratio	Deg Index	Expected
1 day	NSP	269	262	14600	0.5117	26	1.569	1.087	Female mixed?
	SP	3.1871	2.2535	113	2.1256	106	0.708	0.708	Female mixed?
2 day	NSP	33	28	1400	0.0027	0.14	1.10249	0.857	Female mixed?
	SP	0.615	0.4144	21	0.2468	12	0.674	0.857	Female mixed?
3 day	NSP	187	185	9250	0.0037	0	0.807	0.807	Female mixed?
	SP	0.0037	0.0029	0				0.807	No profile

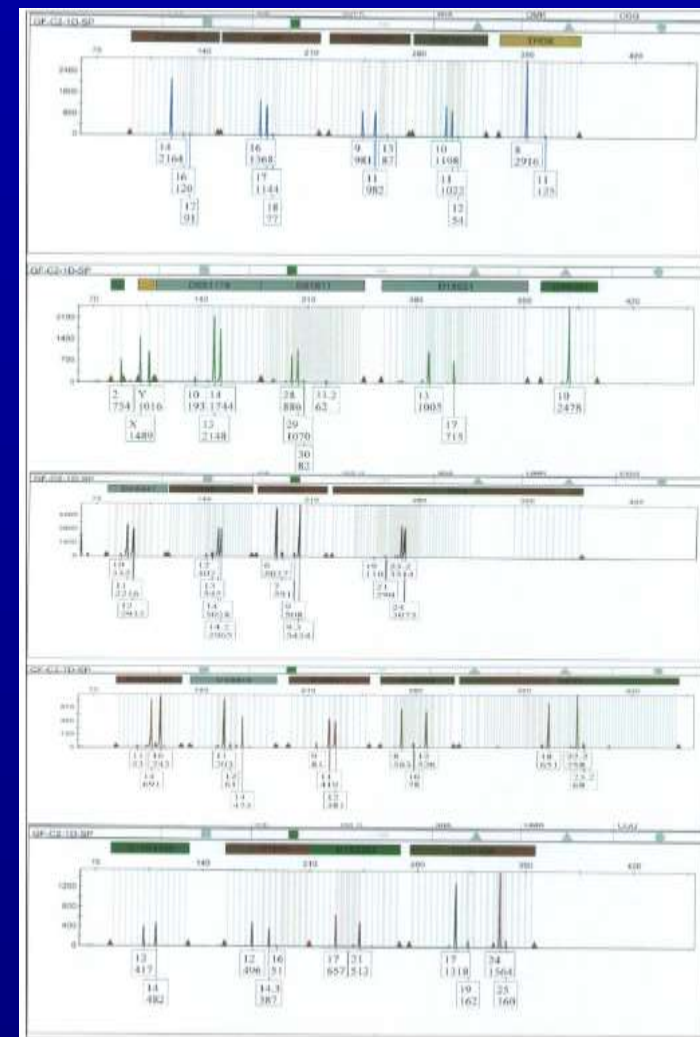
Accuracy of profiles confirmed by comparison to reference profiles

Yfiler Plus									
Post coital Sample	Fraction	T. Large Autosomal (ng)	T. Small Autosomal (ng)	Total Human (ng in 50µl)	T. Y (ng)	Total Male (ng in 50µl)	M:F Ratio	Deg Index	Expected
1 day	NSP	269	262	14600	0.5117	26	1.569	1.087	male
	SP	3.1871	2.2535	113	2.1256	106	0.708	0.708	male
2 day	NSP	33	28	1400	0.0027	0.14	1.10249	0.857	male?
	SP	0.615	0.4144	21	0.2468	12	0.674	0.674	male
3 day	NSP	187	185	9250	0.0037	0	0.807	0.807	no profile
	SP	0.0037	0.0029	0				0.807	no profile

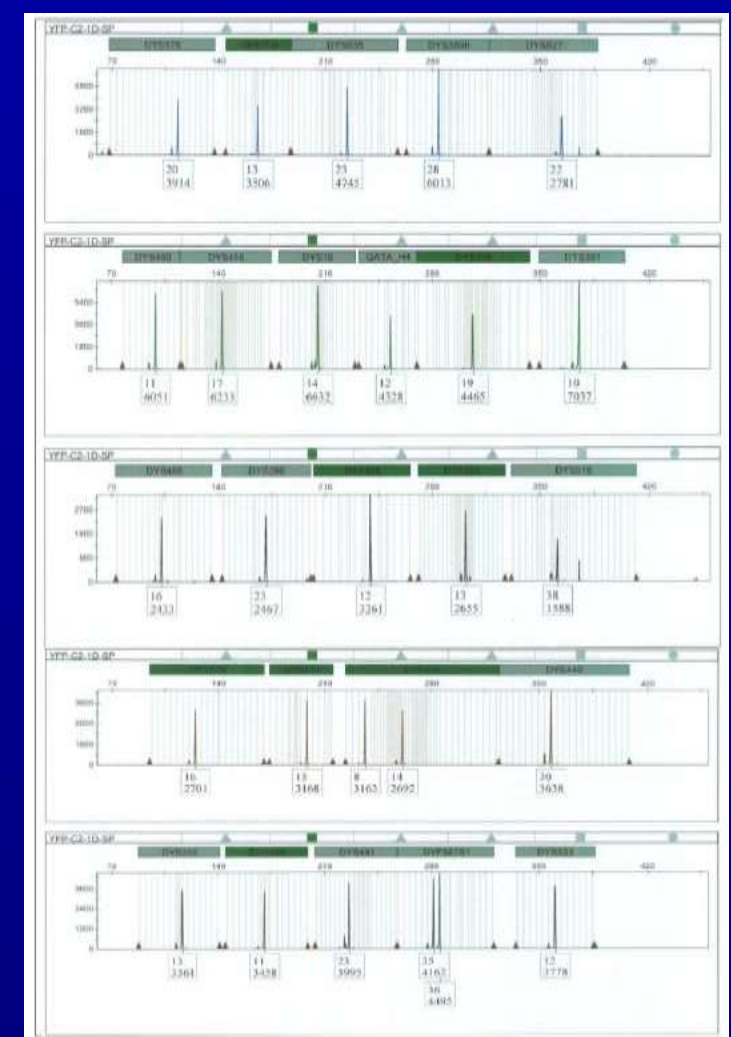
- GlobalFiler™ (Autosomal STRs) – *top panel*
 - Non-sperm fractions (NSP): female profile only
 - 1 day sperm fraction (SP): M/F mixture, major male, very minor female profile
 - 2 day sperm fraction: M/F mixture (~1:1)
 - 3 day sperm fraction: no profile (no male quant; extremely low level human quant value ~0)
- Yfiler® Plus (Y-STRs) – *bottom panel*
 - Full male profiles in 1 day and 2 day sperm fractions (also 1 day NSP)
 - No male profile in 3 day sperm (no male quant, so negative quant = no result)



GlobalFiler™ 1 day Non-sperm Full female profile



GlobalFiler™ 1 day Sperm Full male profile



Yfiler® Plus 1 day Sperm Full male profile

CONCLUSIONS

1. Quantifiler® Trio
 - Excellent correlation between male quantitation and profile recovery
 - Negative quantitation means negative/unusable Yfiler® Plus
2. Quality Index
 - Told us something about our samples (no degradation)
 - Male component "masked" in non-differential extracts; may not completely indicate quality of male DNA component
3. Yfiler® Plus
 - If >100pg male DNA, mostly full profiles
 - Results obtained even with extremely high quantities of background female DNA
 - Many 'usable' profiles with F/M ratios ranging from 333:1 to 100,000:1

ACKNOWLEDGEMENTS

Portions of this work were supported by Award # 2009-DN-BX-0023, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice. We would like to acknowledge Pat Speck and the UTHSC team.

We would also like to acknowledge the Life Technologies team: Jonathan Tabak, Andrea Carbonaro, Sheri Olson, Jeff Sailus, Joanne Sgueglia, Lisa Calandro, Ariana Wheaton, Melissa (a.k.a. Missy) Kotkin, and Shelly Guerrero.

We would also like to acknowledge members of the NCFS staff: Hayley O'Brien and Kelsey Neary for their assistance with population data and data review.