

# Y-Screening and Direct Amplification of Sexual Assault Evidence Kit Samples



October 31, 2016

Presented by:

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# Promega Corporation

## *Y-Screening and Direct Amplification of Sexual Assault Evidence Kit Samples*

*Anupama Gopalakrishnan*

# Casework Direct Kit, Custom

The Casework Direct Kit is used for rapid processing of swabs from casework samples or cuttings of sexual assault swabs and stained clothing prior to quantification of human DNA using the PowerQuant<sup>®</sup> or Plexor<sup>®</sup> HY System and amplification of normalized template using PowerPlex<sup>®</sup> Systems for human STR genotyping.

This kit allows low template samples to be **rapidly** processed, quantified, and amplified with **minimal loss** of DNA.

- For touch DNA or low template casework samples
- For Y-screening applications



# Product Configuration



**Kit Size: 250 reactions**

## Materials Supplied in Kit:

Component	Quantity
Casework Direct Reagent , 100ml	1
1-Thioglycerol, 75 $\mu$ l	1
5X AmpSolution™ Reagent, 500 $\mu$ l	1
Water, Amplification Grade, 1,250 $\mu$ l	5

## Ethylene-Oxide Treated Materials Available Separately:

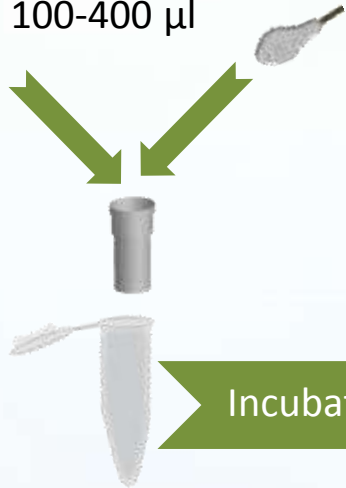
- CW Spin Baskets (50/pk)
- CW Microfuge Tubes (50/pk)

**Available for sale in Dec 2016**

# Simple 2-step protocol



Casework Direct  
Solution, 100-400  $\mu$ l



Incubate at 70°C/30'

Centrifuge for 5 min

discard

2 $\mu$ l

$\leq 15\mu$ l

Quantification using PowerQuant<sup>®</sup>  
System or Plexor<sup>®</sup> HY

STR amplification using  
PowerPlex<sup>®</sup> Systems\*

No Wash  
Steps

=

Minimal  
potential  
for DNA  
loss

The Power to Solve... *from sample to analysis*

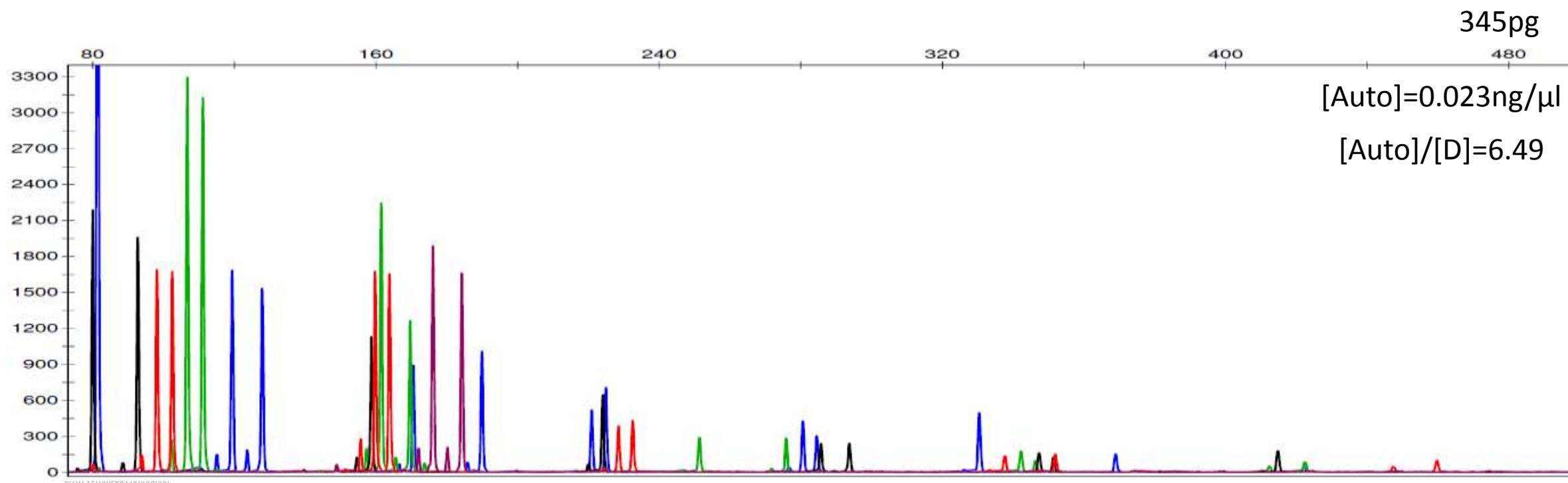
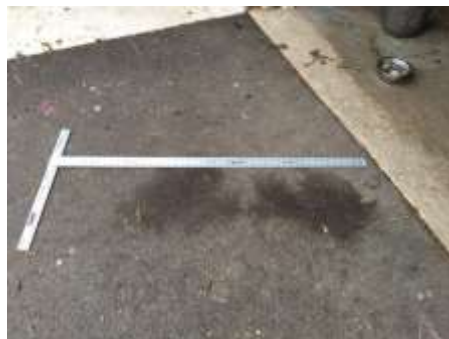
# Compatibility of Casework Direct Lysate

Quantification System	1X AmpSolution Requirement
PowerQuant® System	No
Plexor® HY System	Yes

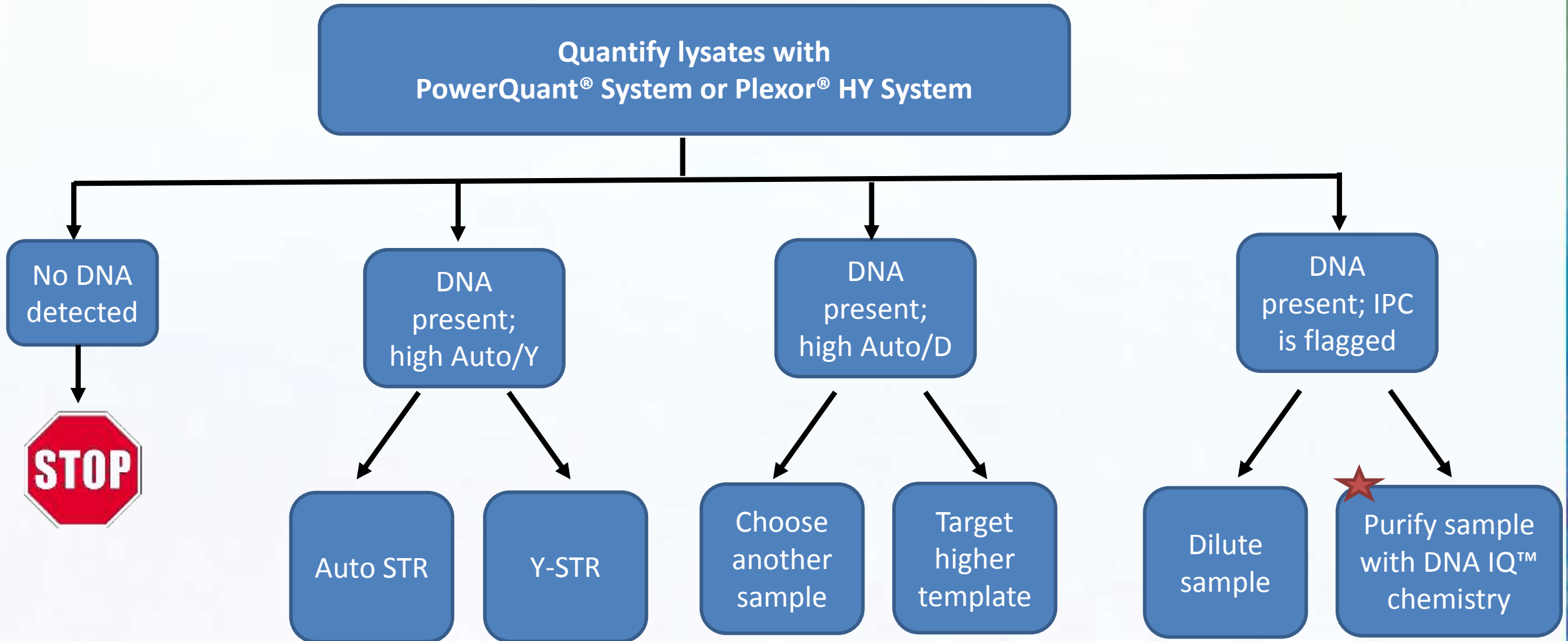
STR System	0.5X AmpSolution Requirement
PowerPlex® Fusion 6C	No
PowerPlex® Fusion	No
PowerPlex® 21	No
PowerPlex® ESX 16/ 17 Fast	Yes
PowerPlex® ESI 16/ 17 Fast	Yes
PowerPlex® Y23*	No (15µl max vol)

# Mock Casework Sample

Blood on driveway  
Swabbed 5 days later  
Exposed to sun and rain



# Decision tree





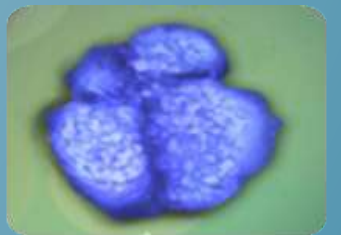
# **Y-SCREENING AND DIRECT AMPLIFICATION OF SEXUAL ASSAULT SAMPLES**

*A Caseworking Laboratory's Perspective*

Amy McGuckian, MSFS, F-ABC  
Technical Leader  
Palm Beach County Sheriff's Office

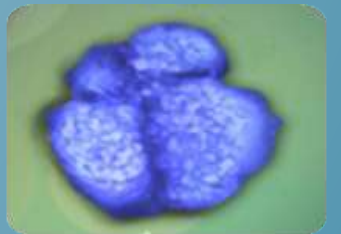
**DISCUSSION  
POINTS:**

- *Why Y-Screen?*
- *Palm Beach County Sheriff's Office study and conclusions*
- *Proposed workflow*



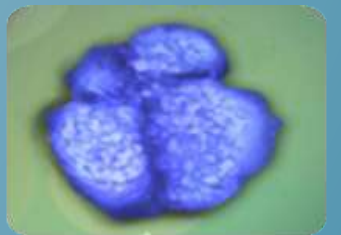
# WHY Y-SCREEN?

- *Conventional screening is laborious and time consuming*
- *Conventional screening results give analysts little predictive power as to what the DNA profile may look like*
- *An absence of seminal fluid/sperm does not preclude the presence of male DNA (e.g. “touch” cases)*
- *The recent passage of “test-all” sexual assault kit (SAK) legislation is increasing demands on forensic biology laboratories*
  - *Imposing time limits for laboratory analysis*
  - *Often no additional funding for personnel and other resources*



# WHY PBSO WAS LOOKING AT Y-SCREENING

- *Senate Bill 636 under consideration by law makers*
  - *Signed in to law March 23, 2016*
  - *Into effect July 1, 2016*
    - *Laboratories have 120 days to complete testing on sexual assaults (not just those with SAKs)*
    - *Looking at the data this would equate to roughly a 100% increase in sexual assault case requests*
    - *Provided no funding for additional resources*



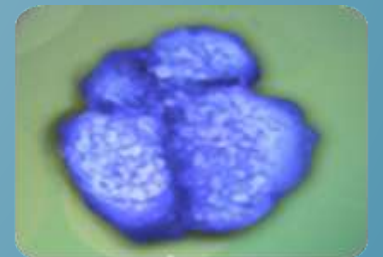


# PROTOTYPE TESTING

- *Began collaboration with Promega in February 2016*
- *Studies conducted in May 2016*

## *Materials and Methods*

- *Prototype Casework Direct Kit*
- *PBSO Methods*
  - *QIAGEN® EZ1 Advanced XL with DNA Investigator Kit*
  - *Promega PowerQuant®*
  - *PowerPlex® Fusion 5C*
  - *PowerPlex® Y23*
  - *AB 3500xl (1.2kV, 24 sec. injection)*



# METHOD

Prototype Casework Direct  
Reagent



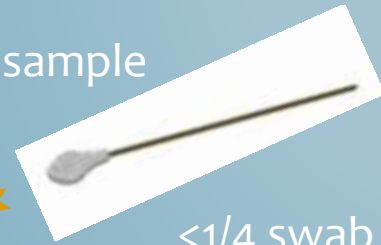
+



1-Thioglycerol

Prototype Casework Direct  
Reagent and 1-Thioglycerol

Add sample



<1/4 swab

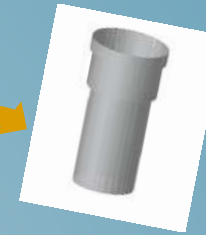


Incubate 30min at 80°C

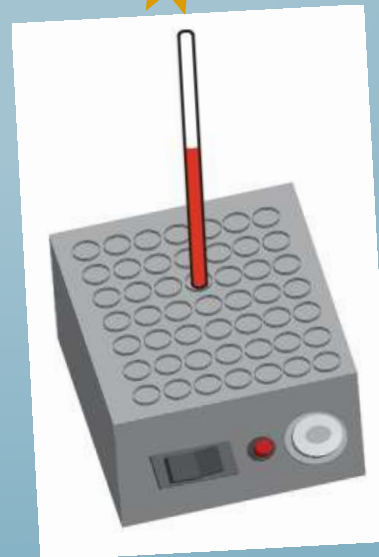
Centrifuge 5 min.



Remove spin basket

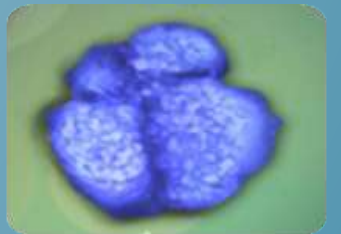


Lysate is ready for  
downstream  
applications



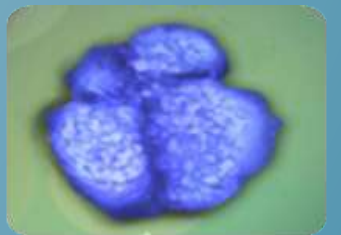
# WORTH NOTING..

- *Extraction method is a whole cell extraction – DNA recovery is not limited by binding capacity of magnetic beads*
  - *Ability to extract more male DNA in the presence of high amounts of female DNA*
- *Also ability to extract micrograms of DNA!*
- *Direct to DNA approach will limit the amount of sample used as secondary sampling may not be required for additional extractions*
- *Consider sample size of cuttings*
  - *Targeted ¼ swab for studies*

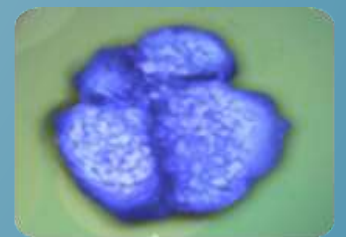
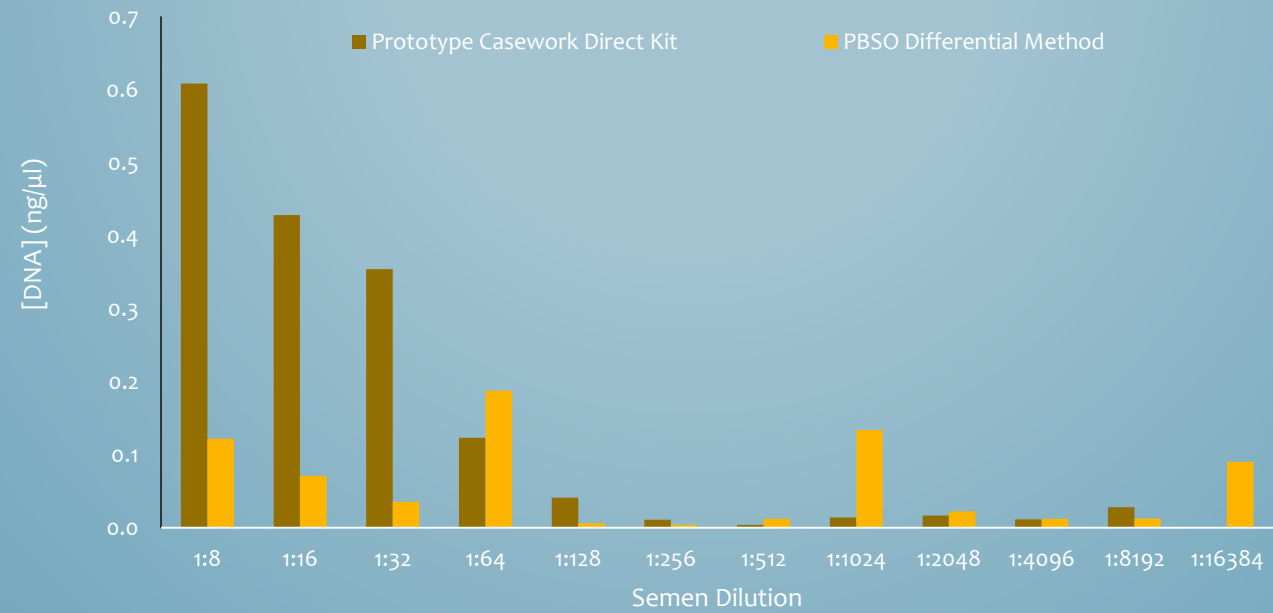
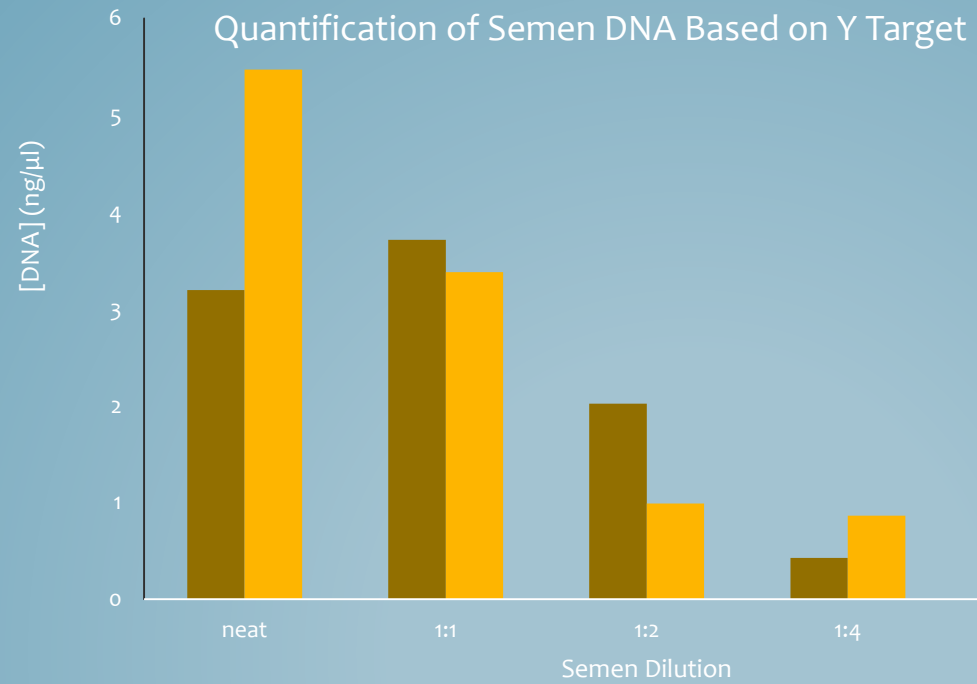


# SENSITIVITY STUDY: MALE DNA IN THE PRESENCE OF HIGH FEMALE DNA

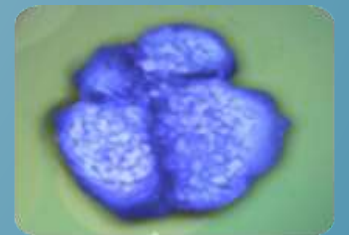
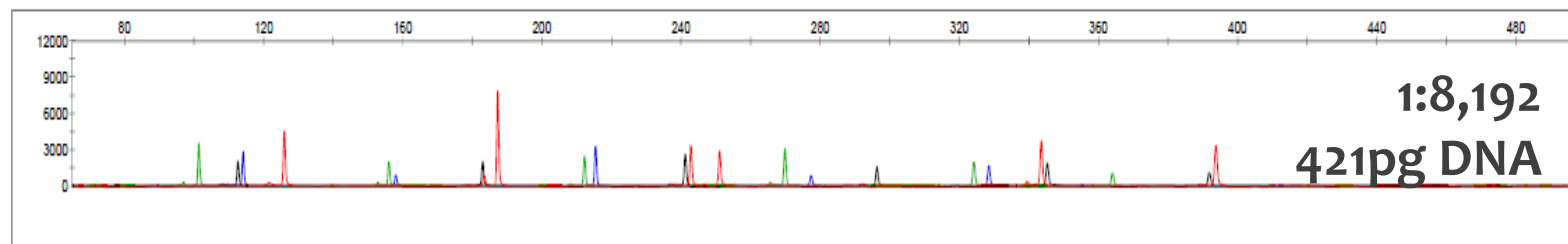
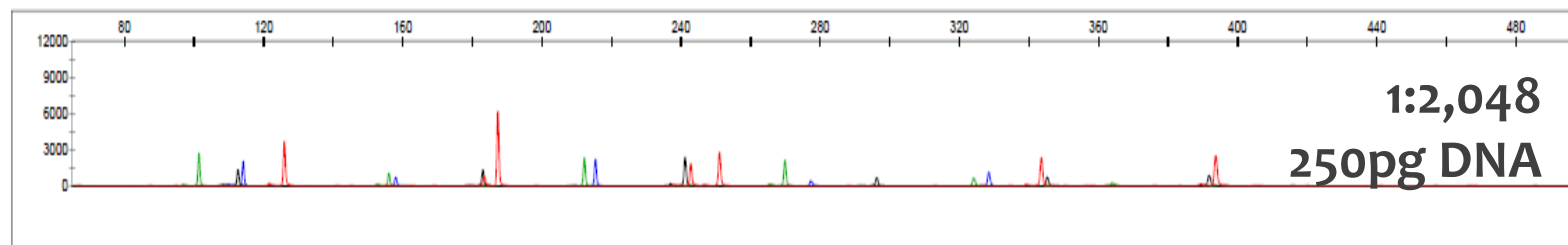
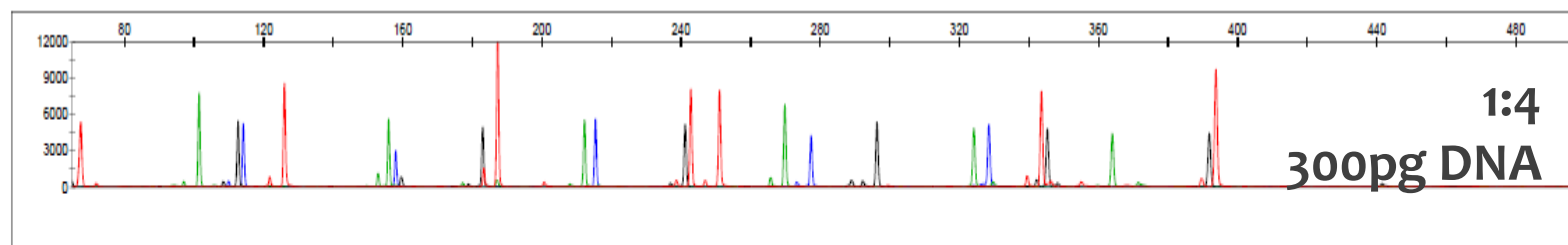
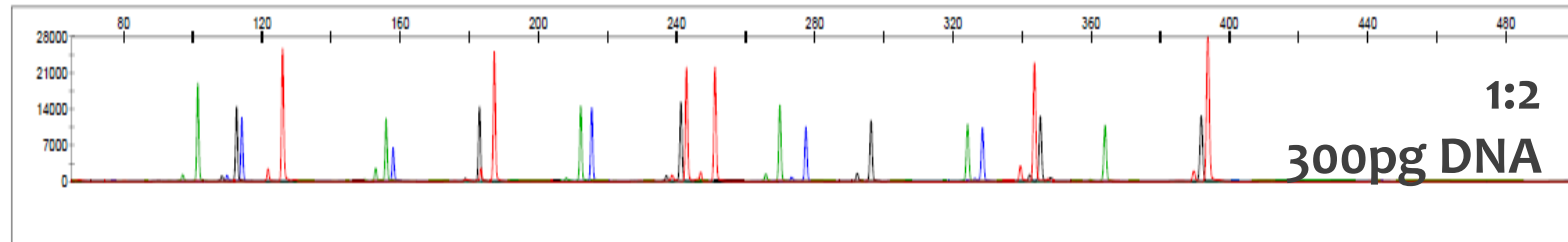
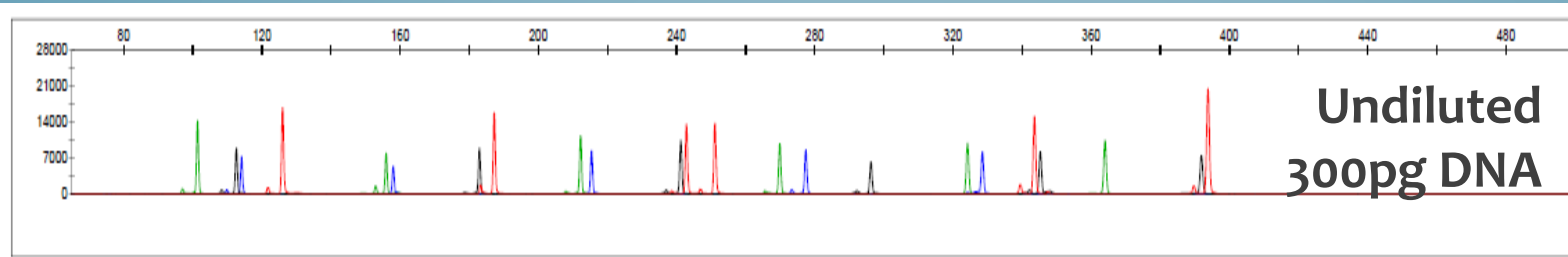
- *Vaginal swabs spotted with 10µl donor semen serially diluted.*
- *¼ of a swab was processed with 400µl Prototype Casework Direct Reagent and laboratory's modified differential extraction method.*
- *Samples were normalized to 300pg template. Normalization was based on autosomal target DNA concentrations for PowerPlex® Fusion and based on Y Target DNA concentrations for PowerPlex® Y23.*



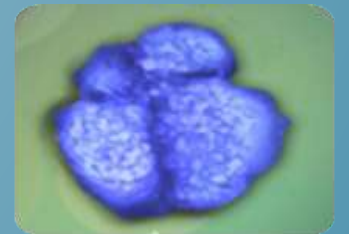
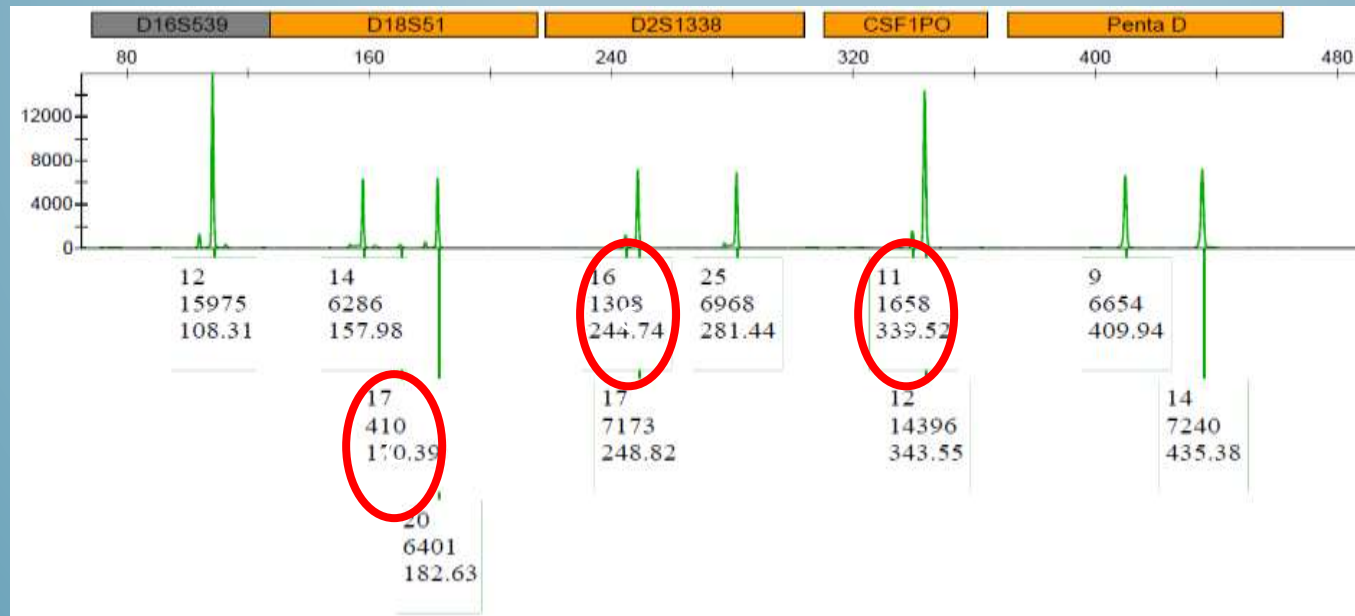
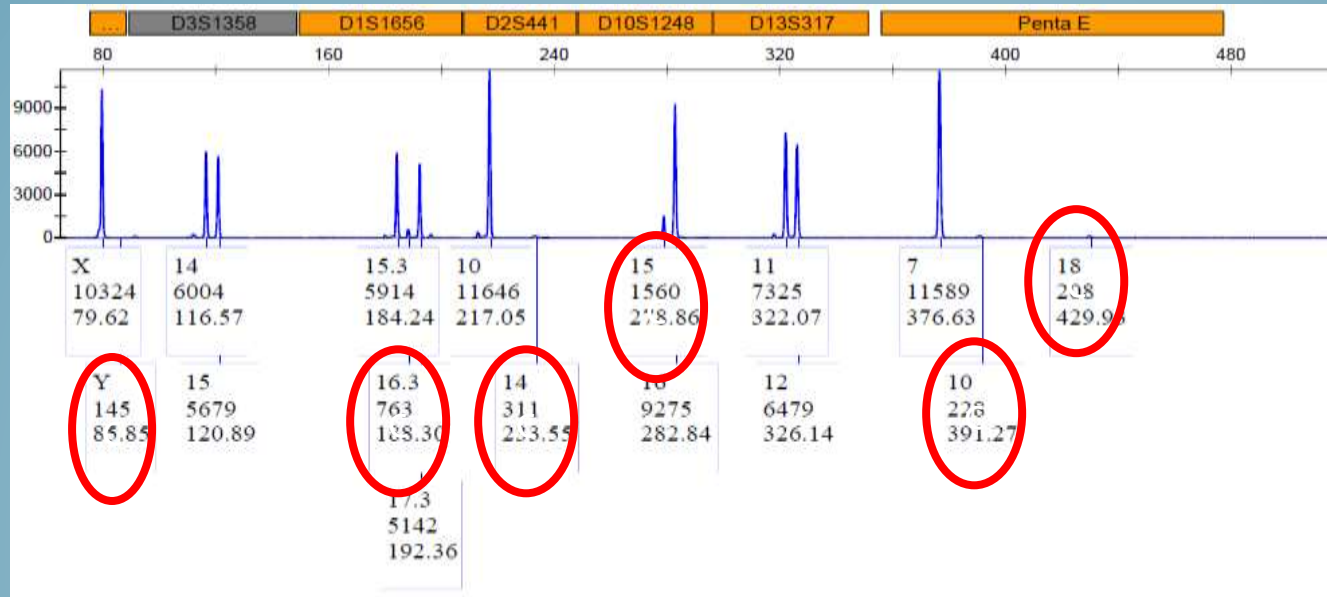




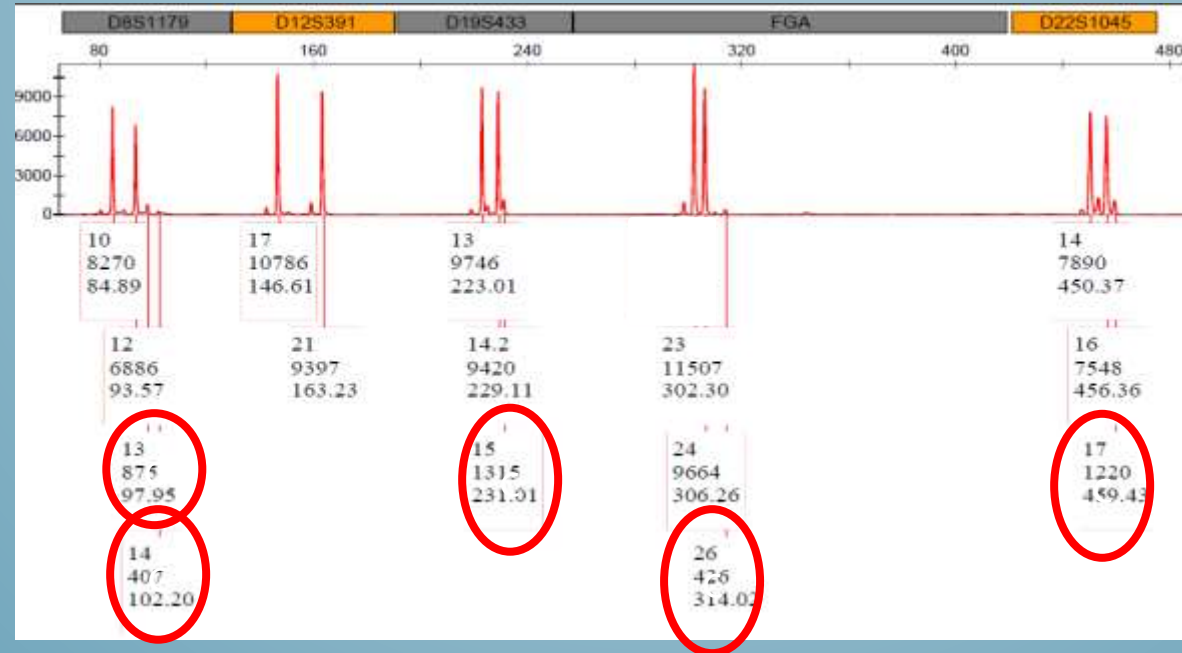
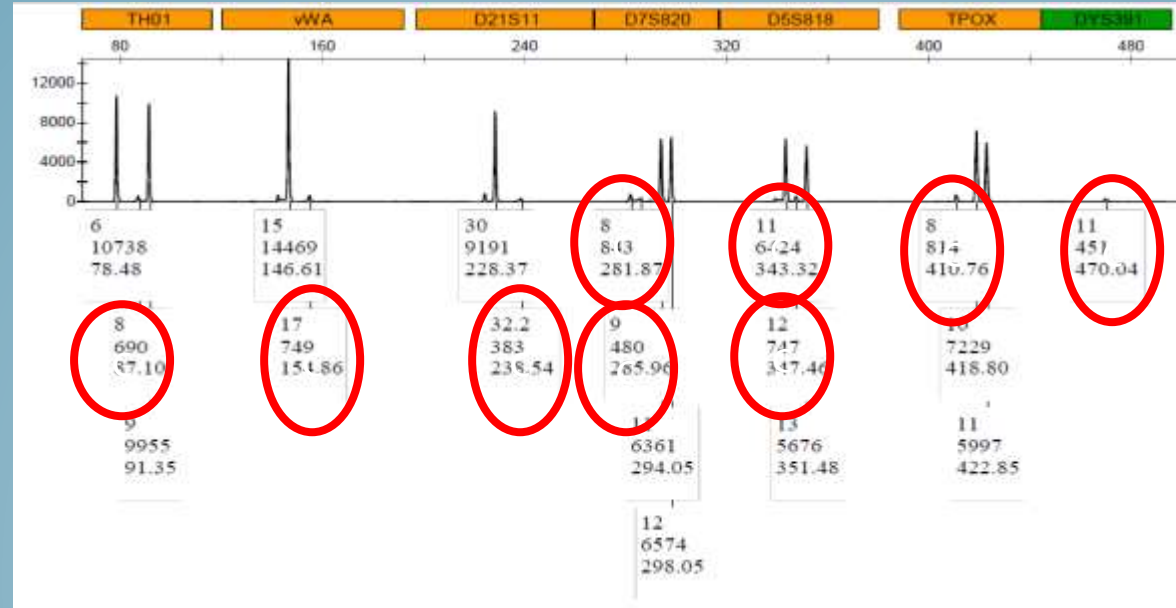
# POWERPLEX® Y23-PROTOTYPE CASEWORK DIRECT KIT



# POWERPLEX® FUSION 1:64-PROTOTYPE CASEWORK DIRECT KIT



# POWERPLEX® FUSION 1:64

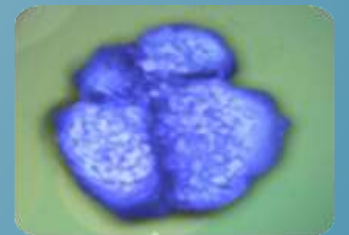




# STUDY: POST-COITAL TIME INTERVALS

Hours Post-Coitus	Prototype Casework Direct Kit			Non-Sperm Fraction			Sperm Fraction		
	Autosomal (ng/μl)	Y (ng/μl)	[Auto] [Y]	Autosomal (ng/μl)	Y (ng/μl)	[Auto] [Y]	Autosomal (ng/μl)	Y (ng/μl)	[Auto] [Y]
5	6.22	1.94	3.2	9.00	0.06	163.0	1.78	1.83	0.97
24	14.59	0.19	77.5	12.89	<0.01	7169.7	0.10	0.05	1.97
48	23.34	0.03	721.5	12.12	<0.01	50840.9	0.04	0.01	6.01
72	22.57	0.02	1050.0	21.59	<0.01	39292.7	0.16	0.04	4.22
96	6.09	0.01	638.3	17.16	N.D.	N.D.	0.14	0.02	8.98

- $\frac{1}{4}$  of a swab was processed with 400μl Prototype Casework Direct Reagent and the laboratory's differential extraction method.
- Samples were normalized to 300pg template. Normalization was based on autosomal target DNA concentrations for PowerPlex® Fusion and based on Y target DNA concentrations for PowerPlex® Y23.

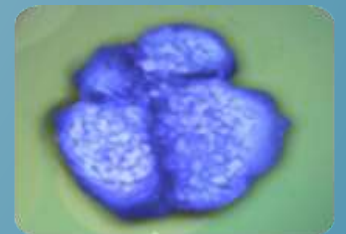


# CORRELATION WITH PRESUMPTIVE SCREENING TESTS

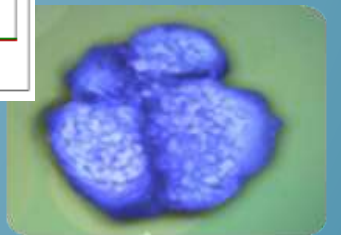
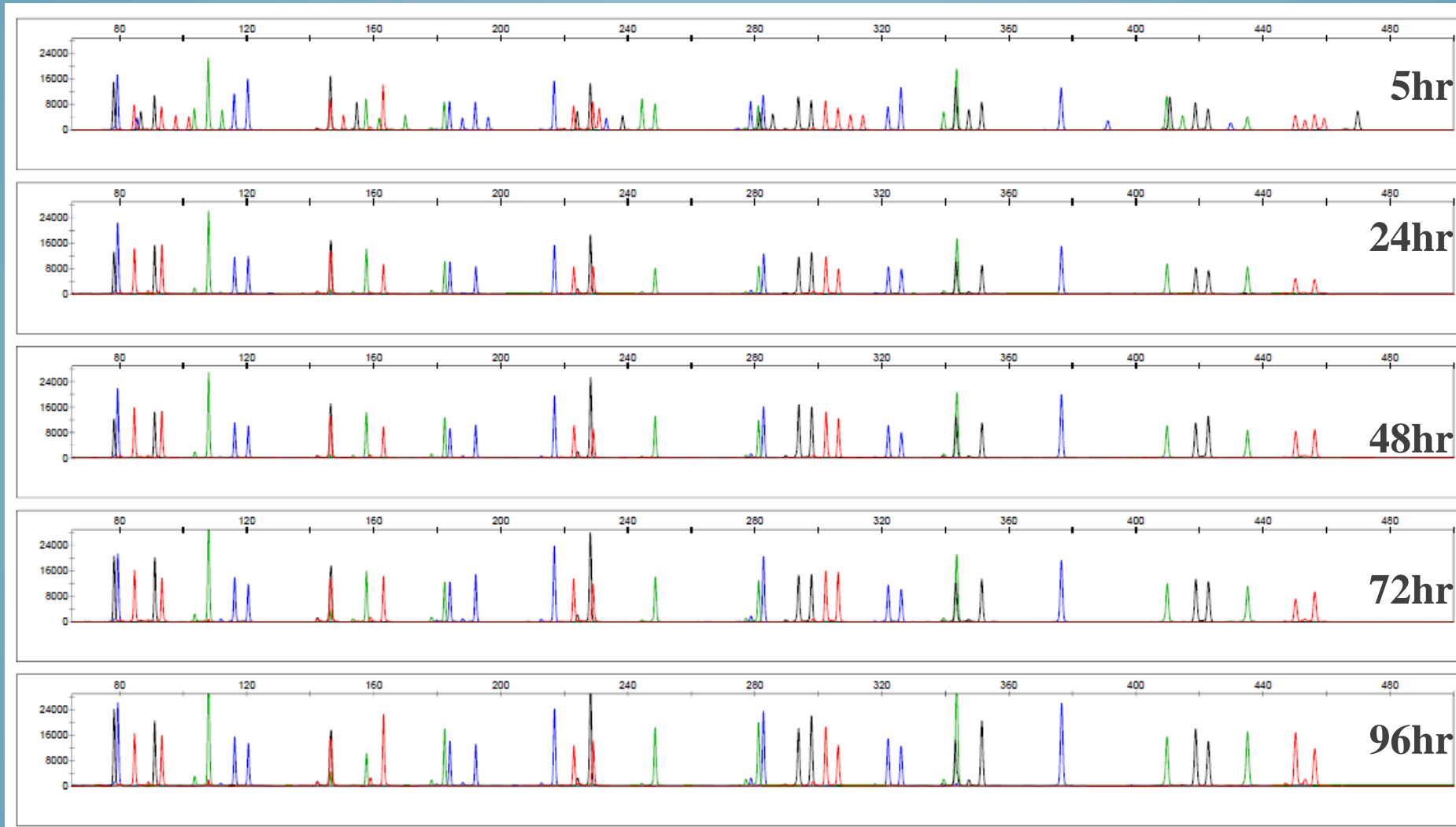
- *Post-coital swabs*

Time Post Coitus	Presumptive Tests		Prototype Casework Direct Kit		Differential Non-Sperm Fraction		Differential Sperm Fraction	
	Acid Phosphatase	Microscopic Evaluation	♂ STR Profile	♂ Y Haplotype	♂ STR Profile	♂ Y Haplotype	♂ STR Profile	♂ Y Haplotype
5hr	+	+	+	+	+	+	+	+
24hr	+	+	Partial	+	-	20/23	+	+
48hr	+	+	one locus	+	-	11/23 Drop-in	+	+
72hr	+	+	-	+	-	14/23 Drop-in	+	+
96hr	-	+	-	+	-	-	+ Drop out	+ Drop in

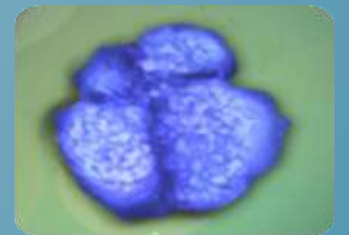
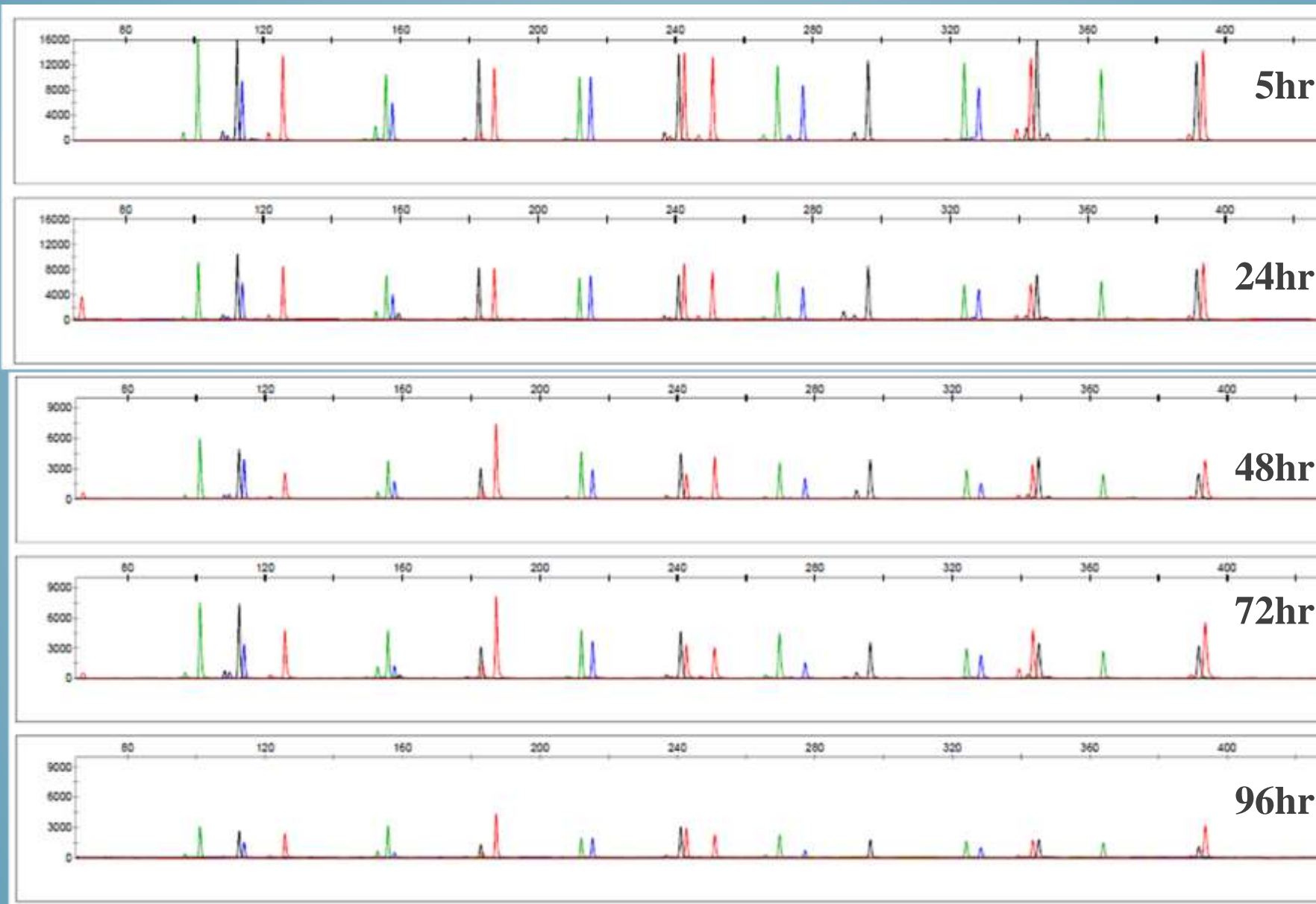
Drop-in = allele detected not belonging to expected profile  
 Drop-out = loss of allele (s) from the expected profile



# PowerPlex® Fusion System-Prototype Casework Direct Kit

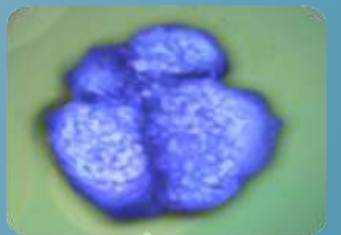


# PowerPlex® Y23 System-Prototype Casework Direct Kit



# STUDY CONCLUSIONS

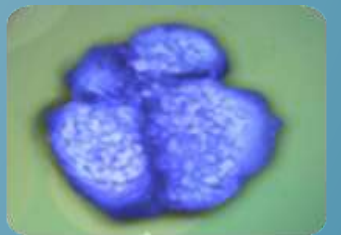
- *Fast*
  - *Lysates can be prepared in less than one hour including sample preparation*
- *Sensitive*
  - *Y23 haplotypes obtained from 1:8192 dilutions with high female background*
  - *Potential to bypass traditional screening methods*
  - *Confidence in stopping analysis*
- *Robust*
  - *Potential to amplify lysates directly for autosomal or Y STRs*





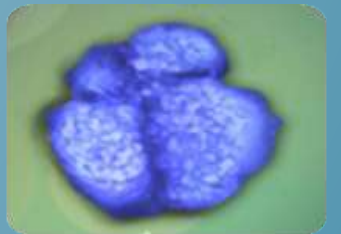
# WORKFLOW PROPOSAL

- *SAKs will be processed as follows:*
  - *Presumptive acid phosphatase screening tests will be performed*
  - *Samples with positive presumptive test results will be extracted with Casework Direct Kit*
    - *Decisions based on sample type (e.g. vaginal swabs), and time of assault to SAK collection will have to be factored in the decision process*
  - *Casework Direct Kit processing will be conducted during evidence screening/ preservation, not by the DNA analyst*
- *Cuttings from relevant swabs from SAKs will be processed with the Casework Direct Kit*
  - *Vaginal and/or related swabs*
  - *Oral swabs (not oral standards)*
  - *Rectal and/or related swabs*
  - *Body swabs*
- **Negative** *extracts for male DNA will terminate the need for additional processing*



# WORKFLOW PROPOSAL

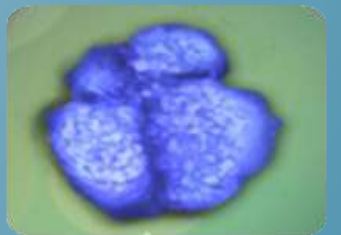
- *Positive samples for male DNA will be assigned to a DNA analyst and either proceed:*
  - *Directly to autosomal amplification*
    - *Can utilize probabilistic genotyping tools such as STRmix to deconvolute mixture (can condition to intimate sample owner)*
  - *To re-extraction with a differential protocol*
    - *When the presence of female DNA is not high enough to obscure the male profile completely, but may cause drop-out*
  - *Directly to Y-STR amplification*
    - *When the ratio of female to male DNA would not generate an interpretable autosomal profile from the male component*



- *Confirming the presence of seminal fluid/sperm will be on a request basis*

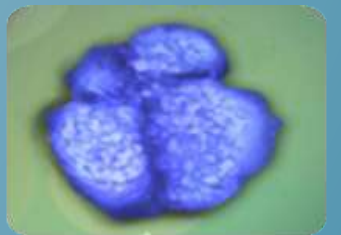
### *Other*

- *Potential to use Casework Direct Kit on other case types and evidence*
  - *Multiple stains on an item*
  - *Touch DNA*



# ADDITIONAL STUDIES

- *Studies to examine different sample sizes*
- *Necessary to test multiple swabs from each area collected? (i.e. test all six vaginal swabs collected, both oral swabs, etc.)*
- *Establish quantification cut-offs to determine when a sample should proceed directly to sample re-extraction with differential protocol to optimize male profile recovery*



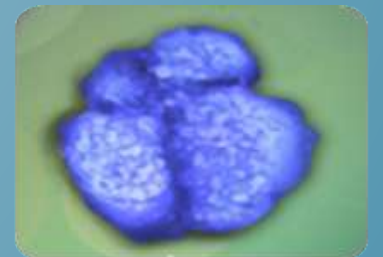
# ACKNOWLEDGEMENTS

## *PBSO*

- *Jerome Remm*
- *Julie Sikorsky*

## *Promega*

- *Anupama Gopalakrishnan*
- *Jeanne Bourdeau-Heller*
- *Bob McLaren*
- *Ann MacPhetridge*





# QUESTIONS

