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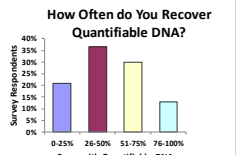
Casework Direct Kit for Direct Amplification of Casework Samples and Y-Screening

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Touch DNA Survey

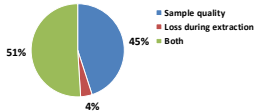
65% survey respondents expected an increase in Touch DNA samples submission

How Often do You Recover Quantifiable DNA?




Cases with Quantifiable DNA	Survey Respondents (%)
0-25%	~18%
26-50%	~35%
51-75%	~28%
76-100%	~10%

Likely Reason for Low Yield



Reason	Percentage
Sample quality	45%
Loss during extraction	51%
Both	4%



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Casework Direct Kit

What

- Rapid processing of swabs from casework samples or cuttings of sexual assault swabs and stained clothing

When

- Prior to DNA quantification and STR genotyping

Why

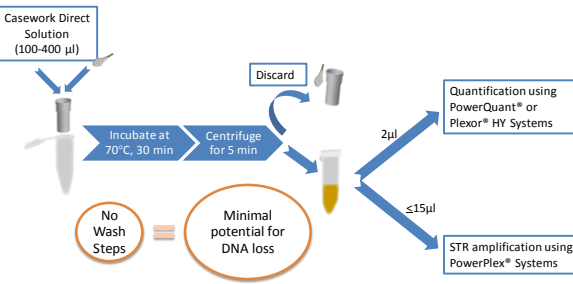
- Allows low template samples to be **rapidly** processed, quantified, and amplified with **minimal loss** of DNA

What For

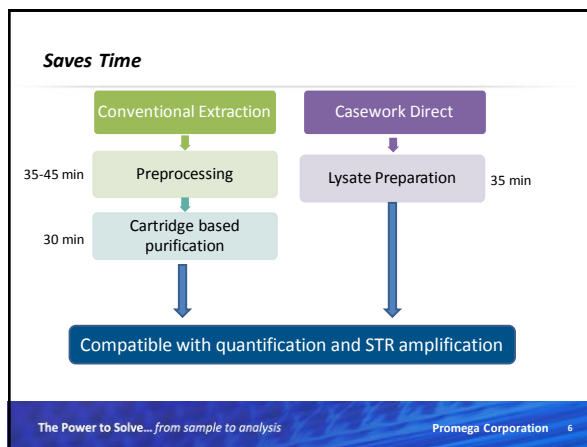
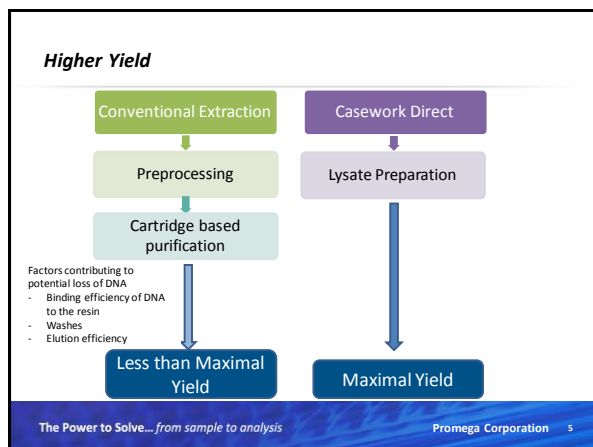
- Touch DNA or low template casework samples
- Y-screening

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Simple, Rapid 2-Step Protocol



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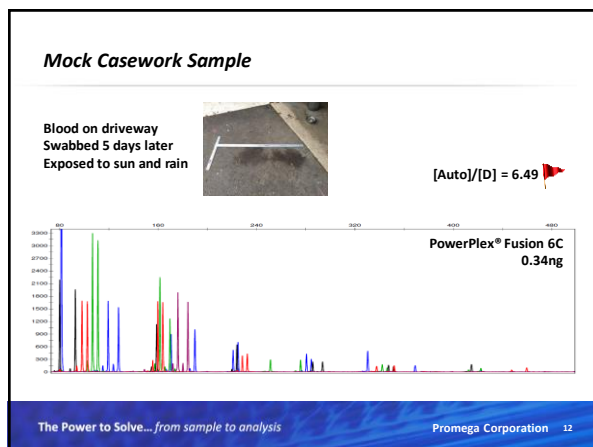
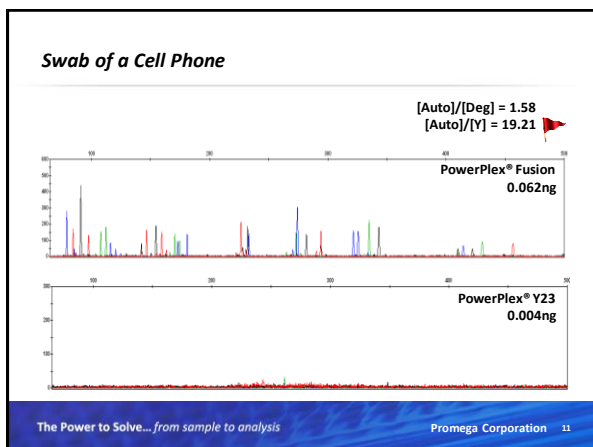
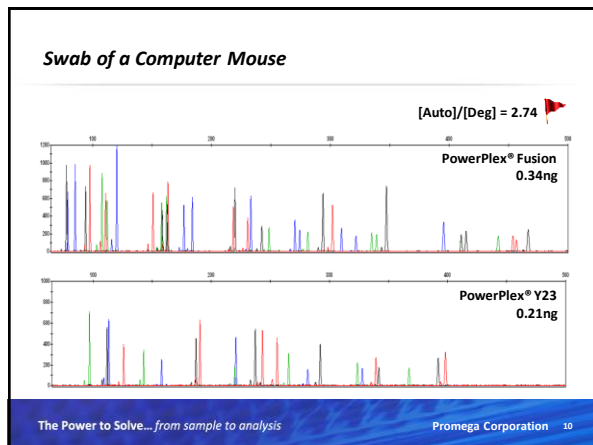
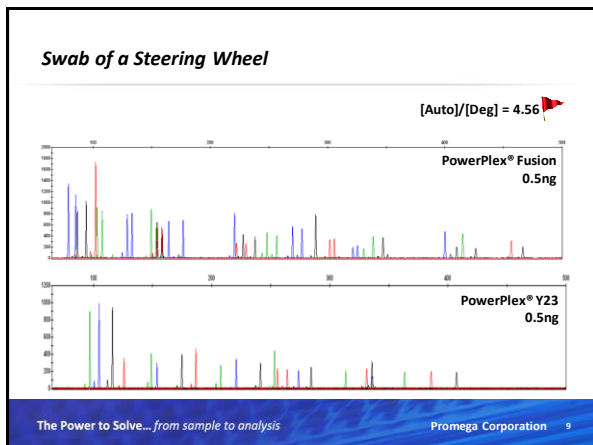
Trace DNA / Touch DNA


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Experimental Design

- Swabbed various surfaces (door handle, steering wheel) with moist cotton swabs
- Processed with 400µl CDS
- Quantification: 2µl of lysate used in PowerQuant® Assay
- STR Amplification: 0.5ng/15µl of lysate was used in PowerPlex Fusion (30 cycles) and Y23 (30 cycles)
- CE: POP-4 ; 3130xl; 3kV 5sec injection
- Data analysis: GeneMapper ID v3.2

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Y-Screening


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Why is Y-screening important?


- 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 sperm/semen does not preclude the presence of male DNA (e.g. "touch" cases)

New SWGDAM Recommendations for SAEK



Scientific Working Group on
DNA Analysis Methods

Recommendations for the
Efficient DNA Processing
Of Sexual Assault Evidence Kits



High Throughput Processing has the following components:

- LIMS
- Standard case approach
- Targeted testing ✓
- Defined workflow ✓
- Automation
- Uniform reporting
- Dedicated resources

www.swgdam.org/publications

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New SWGDAM Recommendations for SAEK


2.1.3 Targeted testing approach: *Direct to DNA*

The serology tests employed by laboratories are less sensitive than modern DNA typing kits. Therefore, DNA typing only the swabs that screen positive in serology tests may miss CODIS-eligible DNA profiles. Rather than use serology to determine which swabs to subject to DNA analysis, a *Direct to DNA* approach is recommended. In the *Direct to DNA* approach, DNA analysis is performed before serology to maximize the chances of obtaining CODIS eligible profiles.

2.1.4 Defined Work Flow

2.1.4.1 Screening for Male DNA via Quantification

1. A quick non-differential extraction technique with a very small portion of one swab (~ 1/8 to 1/16).
2. Quantification method to detect both human and male DNA to determine if male DNA is present.
3. If no male DNA is detected then no further processing of that sample may be necessary.



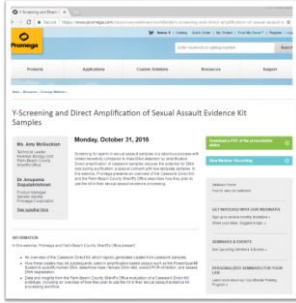
Casework Samples

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Webinar

Legislation resulted in 100% increase in sexual assault case requests with no additional funding.



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Determining the lowest amount of male DNA detected in presence of high female DNA

Sample Preparation

- Vaginal swabs containing female DNA, spotted with decreasing volumes of semen.
- Dilutions used: neat, 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024, 1:2048, 1:4096, 1:8192, 1:16,384.
- Spot 10µl onto swabs, 3 swabs/dilution

CDS Protocol

- Use ~1/4 of swab tip
- Quantify with PowerQuant.
- Amplify and type with PowerPlex Fusion and Y23.

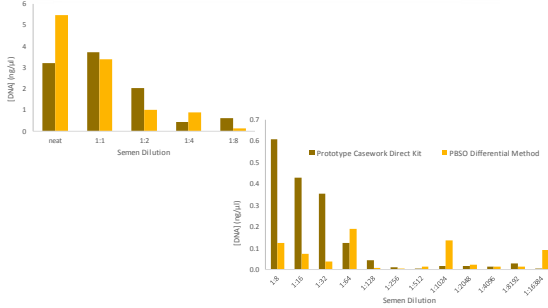
Modified Differential

- Use ~1/4 of swab tip
- Quantify extracts with PowerQuant.
- Compare quantification data from modified differentials to CDS results.

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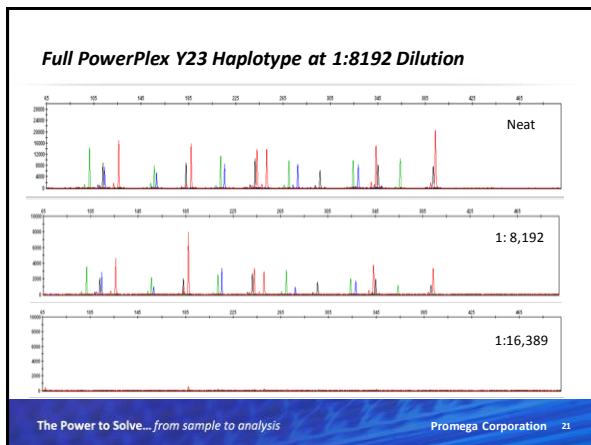
Semen Dilution Series: Quantification Based on Y- Target



Semen Dilution	Prototype Casework Direct Kit (pg/µl)	PISQ Differential Method (pg/µl)
neat	~5.2	~3.2
1:1	~3.8	~3.2
1:2	~2.0	~1.0
1:4	~0.8	~0.4
1:8	~0.4	~0.2
1:16	~0.2	~0.1
1:32	~0.1	~0.05
1:64	~0.05	~0.02
1:128	~0.02	~0.01
1:256	~0.01	~0.005
1:512	~0.005	~0.002
1:1024	~0.002	~0.001
1:2048	~0.001	~0.0005
1:4096	~0.0005	~0.0002
1:8192	~0.0002	~0.0001
1:16384	~0.0001	~0.00005

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Examining the efficacy of Prototype Casework Direct Solution kit on sexual assault swabs collected at different time periods

Sample Preparation

Post-coital swabs collected at different time intervals: 5, 24, 48, 72, and 96 hours.

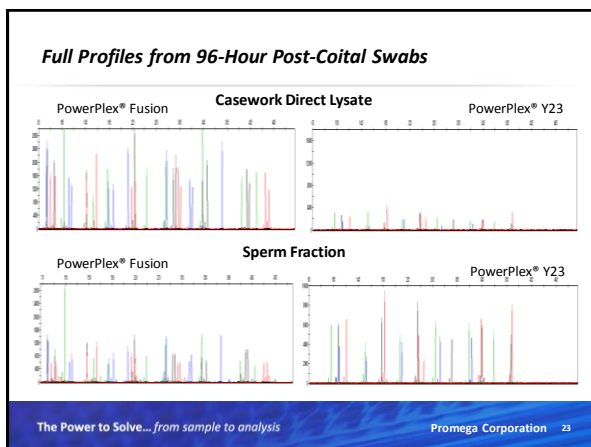
CDS on post-coital samples

- Use ~ ¼ of swab tip
- Quantify with PowerQuant.
- Amplify and type with PowerPlex Fusion and Y23.

Differential extraction on the post-coital swabs

- Use ~ ¼ of swab tip
- Quantify with PowerQuant
- Amplify and type with PowerPlex Fusion and Y23

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Comparison with Presumptive Testing

Hours post-Coitus	Presumptive Tests		Quantification			STR Profile	
	Acid Phosphatase	Microscopic evaluation	[Auto] ng/µl	[Y] ng/µl	[Auto] / [Y]	♂ STR Profile	♂ Y Haplotype
5	+	+	6.22	1.94	3.2	+	+
24	+	+	14.59	0.19	77.5	Partial	+
48	+	+	23.34	0.03	721.5	One locus	+
72	+	+	22.57	0.02	1050.0	-	+
96	-	+	6.09	0.01	638.3	-	+

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Product Details

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Product Configuration

Kit Size: 250 reactions

Materials Supplied in Kit:

Component	Volume	Quantity
Casework Direct Reagent	100ml	1
1-Thioglycerol	75µl	1
5X AmpSolution™ Reagent	500µl	1
Water, Amplification Grade	1,250µl	5

Plastic-ware Available Separately:

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

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Compatible with Quant and STR Amp Systems

Quantification System	1X AmpSolution Requirement	STR System	0.5X AmpSolution Requirement
PowerQuant® System	No	PowerPlex® Fusion 6C	No
Plexor® HY System	Yes	PowerPlex® Fusion	No
		PowerPlex® 21	No
		PowerPlex® ESX/ESI 16/17 Fast	Yes
		PowerPlex® Y23	No (15µl max vol)

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Protocol Online

Rapid Processing of Swabs from Casework Samples Using Casework Direct Kit, Custom

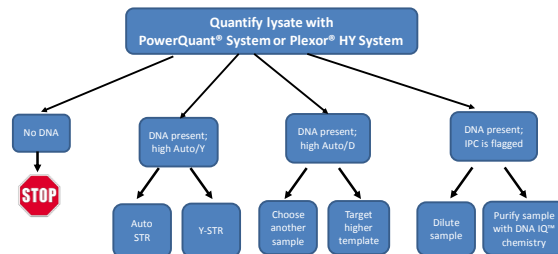
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Summary

- Casework Direct Kit rapidly processes low template samples with minimal loss of DNA.
- Human DNA in the resultant lysate can be quantified & amplified with the PowerPlex® STR Systems.
- Casework Direct Kit is especially useful for Y-screening of sexual assault kits & selection of evidentiary samples for STR testing.

Workflow Decision Tree



Alpha Testers

Bob McLaren
Jeanne Bourdeau-Heller
Doug Storts

Lotte Downey
Ann MacPhetridge

Nick Rogall
Amanda Glebs
Charlie Stolberg
Alyssa TenHarmsel