Normalization and Direct Amplification of Casework Samples

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Abstract #47

Introduction

The sheer abundance of evidentiary samples submitted to forensic labs can be daunting, *which one* contains the necessary information?

Integration of the prototype Casework Direct Solution Kit into a laboratory workflow scheme provides a rapid, cost effective means to generate high quality STR profiles from precious, low-abundance samples with minimal hands on time. This kit rapidly generates lysates from casework swabs and fabric cuttings which are compatible with the PowerQuant® System and PowerPlex® STR Systems.

The PowerQuant® System provides valuable workflow information such as the presence/absence of male DNA, degradation, or potential PCR inhibition, as well as normalization for STR reactions. If no inhibition is detected in the PowerQuant® System it is possible to go directly into a PowerPlex® System STR amplification reaction without any DNA purification, while DNA may be purified from lysates containing inhibitors with Maxwell® 16.
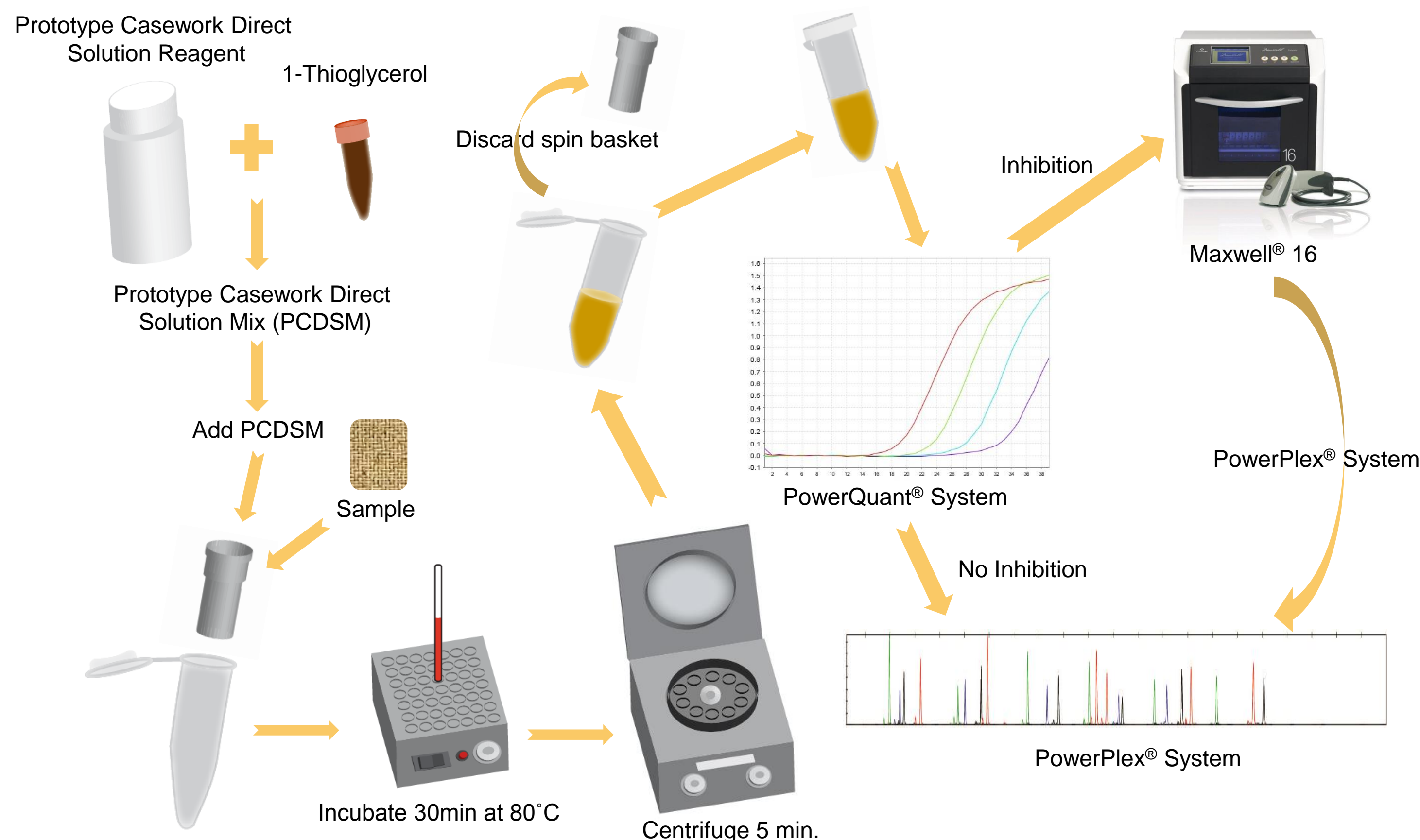
Rapid, Simple Method

Figure 1. Simple Prototype Casework Direct Solution Kit Method.

Sensitivity in Blood Dilution Series

Figure 2. Blood Dilution Series. Donor blood was serially diluted, 100µl of each dilution was pipetted on to a Copan 4N6FLOQSwab™, air dried, and added to 400µl PCDSM. "No Swab" condition is 100µl of dilution pipetted directly into 400µl PCDSM The lysates were tested with both PowerQuant® & PowerPlex® Fusion 6C Systems. Data collected on an Applied Biosystems® 3500xL Genetic Analyzer with a 1.2kV 24sec. injection.

Mock Crime Scene Swabs

Sample	Autosomal Target (ng/µl)	Degradation Target (ng/µl)	Y Target (ng/µl)	IPC Threshold	Auto/Y	Auto/ Degradation
Computer Mouse	0.0456	0.0026	0.0213	Below	2.14	17.45
Keyboard	0.0143	0.0046	0.0002	Below	92.14	3.12
Steering Wheel	0.0769	0.0103	0.0351	Below	2.19	7.48
Bike Handle Bars	0.5196	0.1506	0.3248	Below	1.60	3.45
Rock	0.0074	0.0038	0.0016	Below	4.62	1.96
Saliva + TA	1.4667	ND	2.192	At or Above	0.69	-
Saliva + TA (Purified)	10.6831	8.6382	9.3242	Below	1.15	1.24

PowerPlex® Fusion System
- Computer Mouse (500pg)
- Keyboard (215pg)
- Steering Wheel (500pg)
- Bike Handle Bars (500pg)
- Rock (111pg)
- Saliva + TA (500pg)

PowerPlex® Y23 System
- Computer Mouse (266pg)
- Keyboard (2.5pg)
- Steering Wheel (439pg)
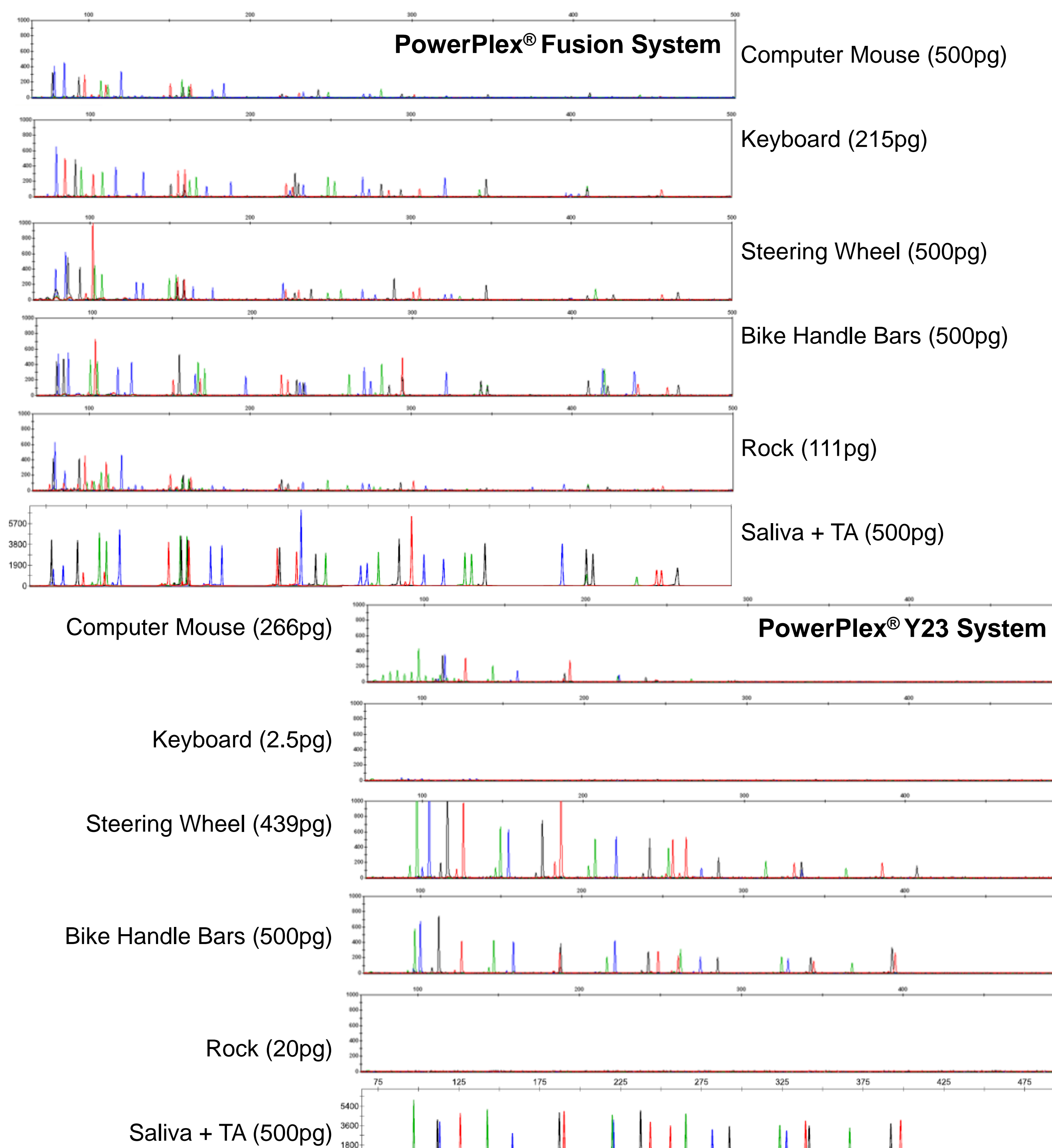- Bike Handle Bars (500pg)
- Rock (20pg)
- Saliva + TA (500pg)

Figure 3. Mock Crime Scene. Various surfaces were swabbed with moist Puritan® Cotton Swabs, saliva contained 2.5mg/ml Tannic Acid (TA). Saliva lysate was subsequently purified with Maxwell® 16. Samples were processed in 400µl PCDSM. Data collected on an Applied Biosystems® 3130x/ Genetic Analyzer with a 3kV 5sec. injection.

Pistol Grip

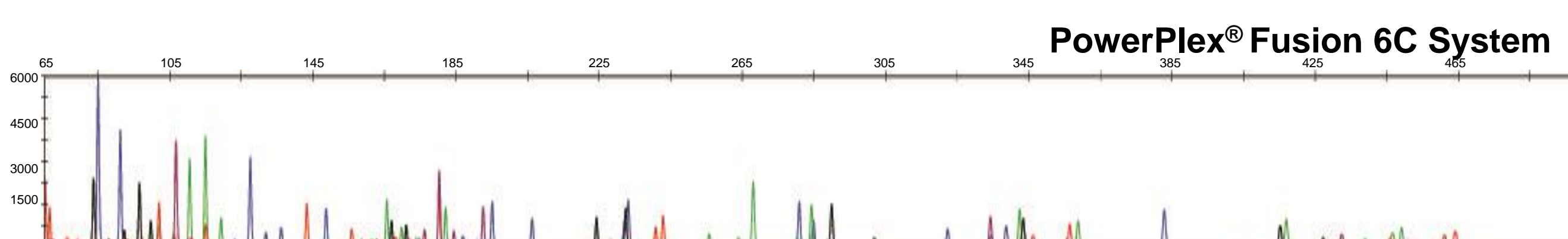| Autosomal Target (ng/µl)	Degradation Target	Y Target (ng/µl)	IPC Threshold	[Auto]/[Y]	[Auto]/[D] Threshold
12.3	7.6	15.2	Below	0.81	Below

PowerPlex® Fusion 6C System

Figure 4. Pistol Grip. Pistol was handled by volunteers and the grip was swabbed with Copan 4N6FLOQSwabs™ which were processed in 400µl PCDSM. Template was normalized to 500pg for the PowerQuant® System. Data collected on an Applied Biosystems® 3500xL Genetic Analyzer with a 1.2kV 24sec. injection.

Semen & Saliva on Black Jeans

PowerPlex® Fusion 6C System — Saliva Side Swab, Semen Side Swab, Post-Swab Cutting
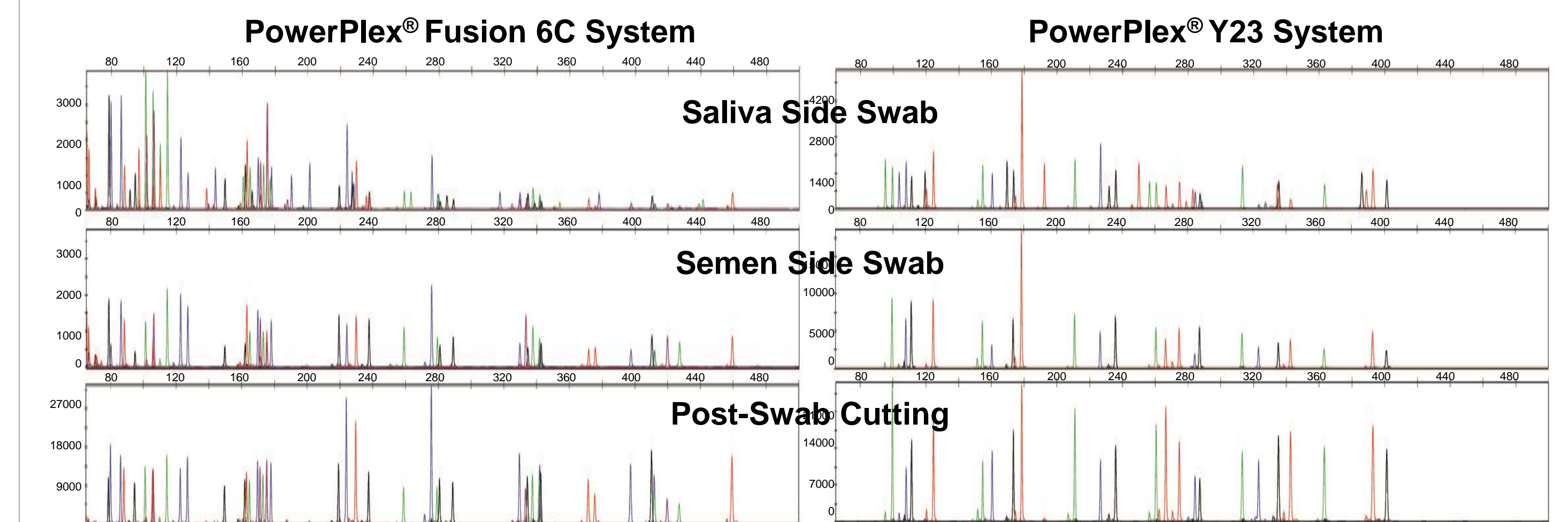
PowerPlex® Y23 System

Figure 4. Saliva & Semen on Black Jeans. Saliva (50µl) was spotted onto one side of a pair of black jeans & air dried, 50µl semen was spotted onto the other side & dried. Each side was swabbed with Copan 4N6FLOQSwabs™ after which a cutting was taken. Samples were processed in 400µl PCDSM. Data collected on an Applied Biosystems® 3500xL Genetic Analyzer with a 1.2kV 24sec. injection.

Prototype Casework Direct Solution Mix is Compatible with Common Forensic Tests

PowerQuant® System Data

Sample	Autosomal Target (pg/µl)	Y Target (pg/µl)	IPC Shift	IPC Threshold
Combur	1350.7	16.9	0.25	Below
Amylase	34.1	1.1	N.D.	At or Above
Black Powder	3017.5	30.8	-1.08	Below
Magnetic Powder	1600.0	19.1	0.56	At or Above

PowerPlex® Fusion 6C System — Combur, Amylase, Black Powder, Magnetic Powder

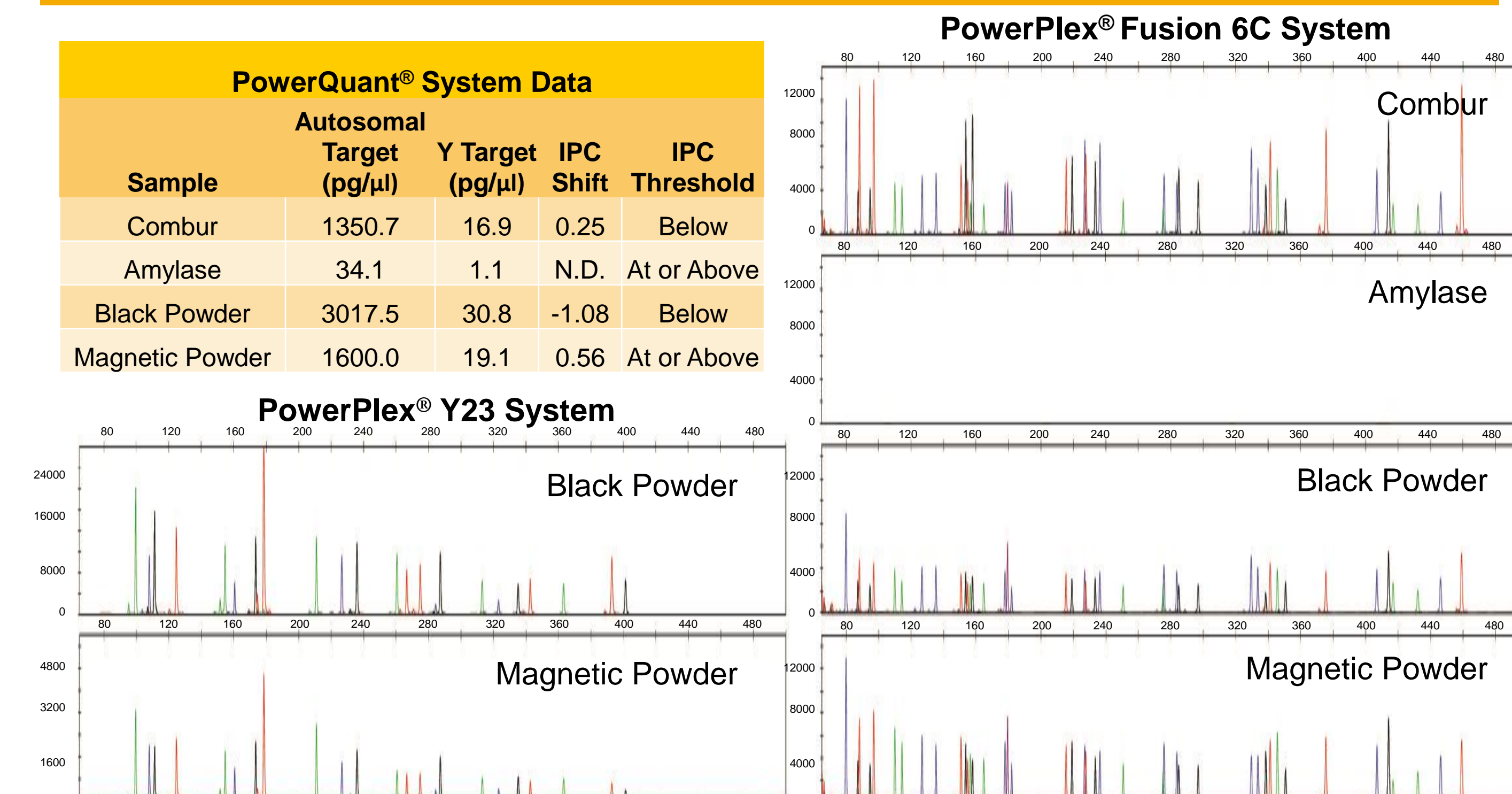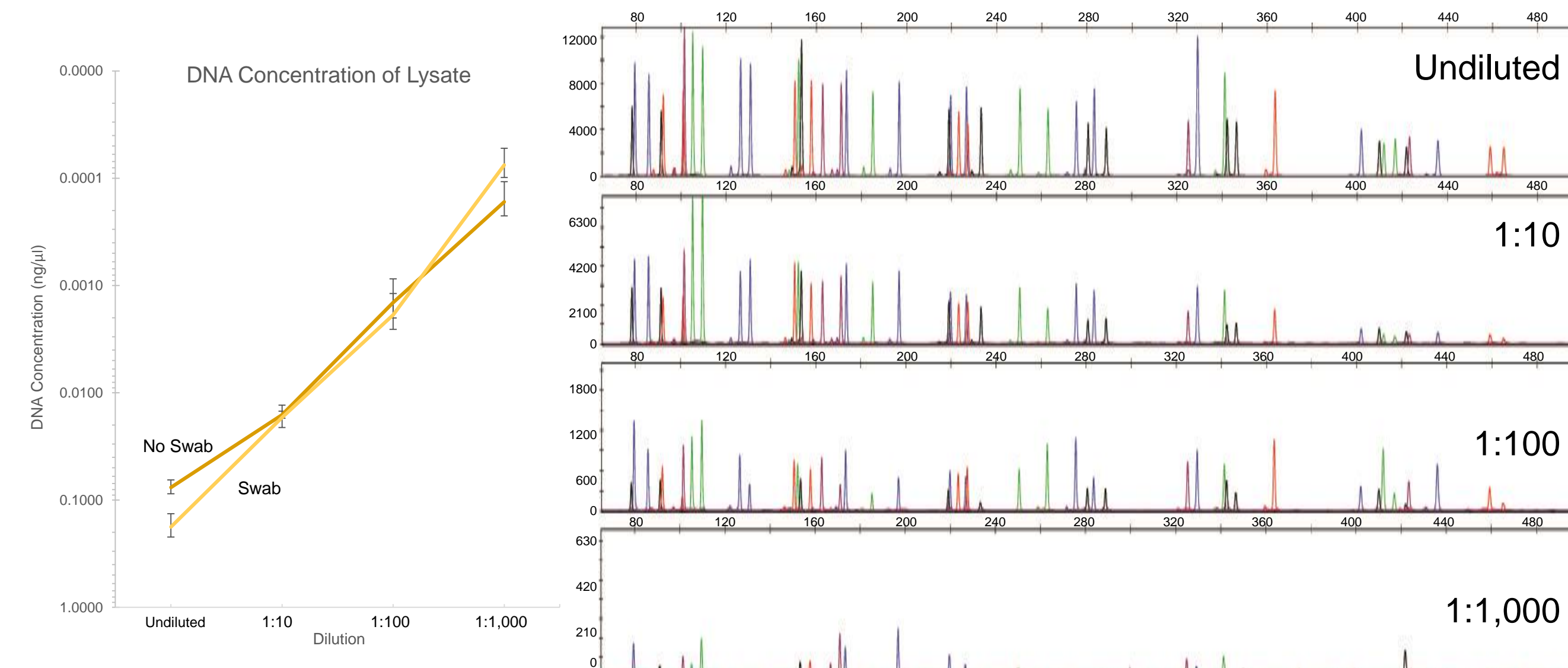PowerPlex® Y23 System — Black Powder, Magnetic Powder

Figure 6. Common Forensic Tests. A mixture of semen & female saliva was added to either serology reagents (Combur or amylase), or fingerprint powders (black or magnetic). Lysates were prepared in 400µl PCDSM. Data collected on an Applied Biosystems® 3500xL Genetic Analyzer with a 1.2kV 24sec. injection.

Summary

Sensitive
- Full profiles in as little as 0.1µl blood in 400µl lysate
- Obtain profiles from a wide variety of swabbed surfaces without need for purification

Compatible with common forensic tests
- Profiles obtained with Combur reagents & fingerprint powders

Streamlines workflow decisions with PowerQuant® System
- Which swab has the needed information?
- Normalize human template from precious casework samples
- Identifies potentially inhibited samples for clean-up using DNA IQ™ Casework Pro Kit for Maxwell® 16.

Available for Sale in December 2016 as "Casework Direct Kit, Custom".

Acknowledgements

www.promega.com
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