From Swabs to Sequencing: Integrating Y-Screening and NGS into Sexual Assault Evidence Processing

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INTRODUCTION

The most common type of DNA evidence analyzed in crime laboratories are sexual assault kits (SAKs). There is currently a backlog of approximately 50,000 untested SAKs in the United States[1]. Serological screening of SAKs can be time consuming, subjective, and not always a good indicator of which samples may result in successful STR profiles[2]. Y-chromosome specific qPCR (Y-screening) is a quantitative alternative that can provide a more objective and reliable indication of samples that may be more successful in obtaining profiles[3]. This screening method would allow for better decision-making when selecting samples for DNA typing.

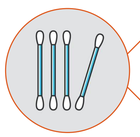
As forensic laboratories continue to integrate advanced sequencing platforms, it is essential that front-end screening methods such as Y-screening remain compatible with evolving downstream technologies. Next Generation Sequencing (NGS) methods are valuable in forensics because they provide a higher level of genetic detail compared to traditional STR analysis, allowing for more accurate identification and mixture interpretation. NGS can simultaneously analyze multiple genetic markers, such as STRs and SNPs, in a single workflow, increasing efficiency and data richness[4]. Evaluating Y screening performance across both capillary electrophoresis (CE) and NGS workflows ensures consistent and reliable results in sexual assault evidence processing.

MATERIALS & METHODS

MOCK & AUTHENTIC SEXUAL ASSAULT SAMPLES

Fluid Mixtures on Swabs

- Semen: Saliva (F)
- Semen: Saliva (M)
- Semen: Blood (M)



Mock Sexual Assault Swabs

- Semen dilutions on **vaginal** swabs
- Semen dilutions on **buccal (M)** swabs
- Semen dilutions on **epithelial (M)** swabs

Authentic Post-Coital Swabs

- 6 hours
- 12 hours
- 48 hours

RESULTS & DISCUSSION

Table 1. Pellet Screening and Pellet Differential Extraction Results. Green boxes indicate good profile qualities (high quantification and allele recovery), while yellow indicates intermediate, and red indicates poor. Mixture Indices of Male-Male mixtures are greyed-out because they are not informative.

	Fluid/Swab	Mixture Ratio/Semen Dilution/TCI	Pellet Screening		Pellet Differential			
					Quantification		Profiling	
			Y DNA Concentration (ng/µL)	Mixture Index	F2 Y DNA Concentration (ng/µL)	F2 Mixture Index	F2 Unique POI a-STR Allele Recovery CE (%)	F2 Unique POI a-STR Allele Recovery NGS (%)
Fluid Mixture Control	Semen (M): Saliva (F)	1:1	4.8692	0.97	3.1956	0.69	100	100
		20:1	7.0176	0.92	2.5077	0.72	100	100
		50:1	5.2653	0.84	2.0157	0.80	100	100
		1:20	0.3584	1.87	0.1570	0.72	100	100
		1:50	0.1280	2.79	0.0713	0.76	100	100
	Semen (M): Saliva (M)	1:1	6.2253	1.00	2.6881	0.72	100	100
		20:1	1.3427	0.38	4.2356	0.74	100	100
		50:1	0.5237	0.67	2.5139	0.65	100	100
		1:20	0.1306	0.44	0.0864	0.68	100	100
		1:50	1.2032	0.78	0.1423	0.84	100	100
	Semen (M): Blood (M)	1:1	0.4776	0.40	6.6149	0.70	100	100
		20:1	4.9514	0.76	3.8724	0.71	100	100
		50:1	3.4732	0.73	3.1710	0.72	100	100
		1:20	0.0138	5.47	0.1515	0.85	100	100
		1:50	0.8010	1.07	0.0675	0.84	100	100
Mock Sexual Assault	Vaginal	1:3	18.6094	1.88	8.6186	0.75	100	100
		1:15	6.5730	10.39	1.1939	0.68	100	100
		1:60	1.3635	10.02	0.1597	0.77	100	100
		1:1500	0.0009	>1000	0.0087	25.07	50	60
		1:7500	0.0017	>1000	0.0020	412.98	0	0
	Buccal (M)	1:3	28.4195	0.94	6.0638	0.72	100	100
		1:15	5.8105	0.91	1.2972	0.72	100	100
		1:60	33.2952	1.11	0.6826	0.72	100	100
		1:1500	26.2402	1.17	0.0065	0.85	87.5	86
		1:7500	10.1876	0.90	0.0088	0.94	41.67	50
		1:37500	33.6854	1.00	0.0122	1.08	0	5
	Epithelial (M)	1:3	1.9781	0.37	1.8699	0.69	100	100
		1:15	0.2955	0.66	0.5228	0.55	100	100
		1:60	0.2591	0.61	0.0666	0.76	100	100
		1:1500	0.0120	0.51	0.0010	0.61	10	29
		1:7500	0	0.70	0.0003	0.80	6.67	25
		1:37500	0.0029	0.63	0	UND	3.33	7
Authentic Post Coital	Post Coital	6 hrs	3.3202	4.79	1.8798	0.99	100	100
		12 hrs	5.3837	16.33	1.0737	0.94	100	100
		48 hrs	0.0345	82.58	0.0268	1.26	86.67	100
		48 hrs	0.0646	609.74	0.0159	7.20	96.67	100

Table 2. Swab Screening and Differential Extraction Results. Green boxes indicate good profile qualities (high quantification and allele recovery), while yellow indicates intermediate, and red indicates poor.

	Fluid/Swab	Mixture Ratio/Semen Dilution/TCI	Swab Screening	Differential Extraction			
				Quantification		Profiling	
			Y DNA Concentration (ng/µL)	F2 Y DNA Concentration (ng/µL)	F2 Mixture Index	F2 Unique POI a-STR Allele Recovery CE (%)	F2 Unique POI a-STR Allele Recovery NGS (%)
Fluid Mixture Control	Semen (M): Saliva (F)	1:1	2.3756	11.4712	0.66	100	100
		20:1	3.0664	19.6707	0.54	100	100
		50:1	8.8665	12.1138	0.70	100	100
		1:20	0.1322	0.3230	0.74	100	100
		1:50	0.1067	0.1252	0.68	100	100
Mock Sexual Assault	Vaginal	1:3	0.8492	9.4892	0.83	100	100
		1:15	0.3870	3.0956	0.91	100	100
		1:60	0.0191	0.3793	0.91	100	100
		1:1500	0.0005	0.0119	89.17	0	3
		1:7500	0.0004	0.0008	409.60	0	0
Authentic Post Coital	Post Coital	6 hrs	0.3463	3.6033	1.03	100	100
		12 hrs	0.0537	2.2161	1.05	100	100
		48 hrs	0.0004	0.0071	8.48	86.67	64
		48 hrs	0.0011	0.0444	4.28	100	100

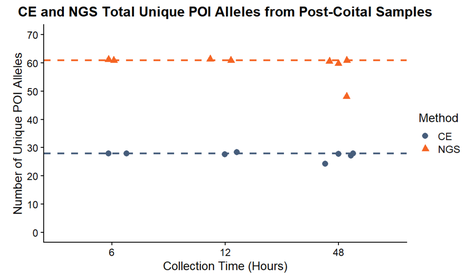
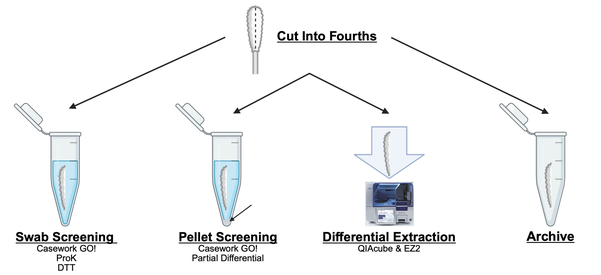


Figure 1. Scatter plot depicting Total Number of Unique Alleles of Semen Donor (POI) from Post-Coital Samples. STR typing completed using F2 fractions from differential extractions. All loci in CE and NGS methods considered. Corresponding color-coded lines represent total expected number of unique alleles from POI.

Table 3. Random Match Probabilities for POI in Post-Coital Samples. RMP's calculated using ArmedXpert™ for CE profiles and MixtureACE™ for NGS profiles. Results are reported on a negative logarithmic scale as a range for each sample type. Two replicates used at 6 and 12 hours, 4 replicates used at 48. To compare overlap, only loci included in both Investigator 24Plex QS and ForenSeq MainstAY included in calculations. To compare total discriminatory power, all loci from respective kit included.

	-log RMP Range			
Sample	CE Total Power	CE Overlap	NGS Overlap	NGS Total Power
6 hrs	29	26	39	50-54
12 hrs	29	26	39	50
48 hrs	18-22	16-20	20-34	31-48

- Y-Screening methods often predicted downstream STR success **(Tables 1 & 2)**.
- Male-male mixtures presented a challenge for Y-Screening, where high Y-Screening values were not always indicative of successful STR profiles **(Table 1)**.
- Profiles from NGS workflow showed high concordance with CE based methods **(Tables 1 & 2)**.
- NGS included more loci than CE by adding autosomal STRs and Y-STRs, increasing the information available for interpretation **(Figures 1 & 2)**.



Y-SCREENING (SWAB AND PELLET SCREENING) CARRIED OUT USING INVESTIGATOR CASEWORK GO! AND QUANTIPLEX PRO

TRADITIONAL DIFFERENTIAL EXTRACTIONS CARRIED OUT WITH QIAGEN QIACUBE CONNECT FX AND EZ2 CONNECT FX

POSITIVE PELLET SCREENINGS UNDERWENT DIFFERENTIAL EXTRACTION WITH QIAGEN QIACUBE CONNECT FX AND EZ2 CONNECT FX

STR TYPING COMPLETED USING:

INVESTIGATOR 24PLEX QS (CE)
FORENSEQ MAINSTAY (NGS)

RMP ANALYSIS COMPLETED USING:
ARMEDXPERT™ (CE)
MIXTUREACE™ (NGS)

CONCLUSIONS

- Integrated front-end screening with multi-platform STR typing enhances the evaluation of sexual assault evidence.
- Incorporation of CE and NGS-based typing platforms offers more efficient and objective forensic workflows.
- RMPs demonstrate increased discriminatory power of NGS at 6 and 12 hours post-coital, with reduced discrimination at 48 hours due to increased mixture complexity.
- Next steps include refining thresholds and applying probabilistic NGS approaches for mixture interpretation.

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