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

Lyndsy Stacy, MS; Joshua Boster, MS; Jennifer Snedeker, PhD; Rachel Houston, PhD

*Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340*

Sam Houston State University

## 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.

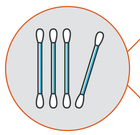
As forensic laboratories continue to integrate advanced sequencing platforms, it is essential that front-end screening methods such as Y-screening remain compatible with evolving downstream technologies. Next Generation Sequencing (NGS) methods are valuable in forensics because they provide a higher 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 |

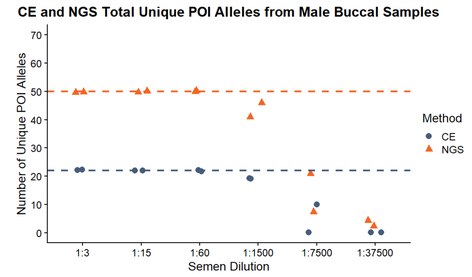



**Figure 1. Scatter plot depicting Total Number of Unique Alleles of Semen Donor (POI) from Male Buccal Samples.** STR typing completed using F2 fractions from differential extractions. All loci in CE and NGS methods considered. Corresponding color-coded lines represent total expected number of unique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

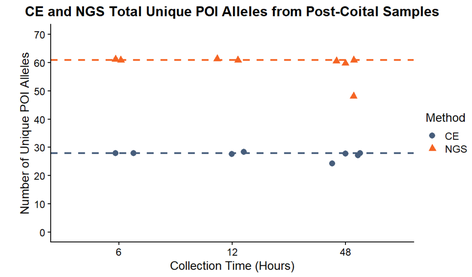



**Figure 2. Scatter plot depicting Total Number of Unique Alleles of Semen Donor (POI) from Post-Coital Samples.** STR typing completed using F2 fractions from differential extractions. All loci in CE and NGS methods considered. Corresponding color-coded lines represent total expected number of unique alleles from POI.

**Table 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 |

- 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)**.

## REFERENCES

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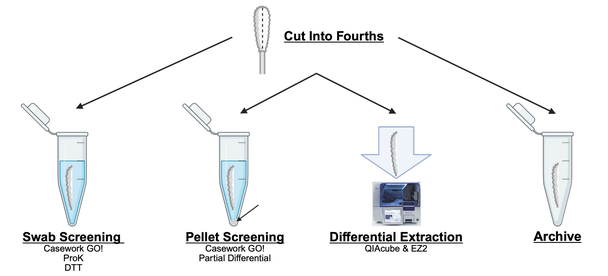
4. De Knijff, P. (2019). From next generation sequencing to now generation sequencing in forensics. Forensic Science International: Genetics, 38, 175–180. https://doi.org/10.1016/j.fsigen.2018.10.017

## MATERIALS & METHODS




**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)**

## 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.
- Next steps include refining thresholds and applying probabilistic NGS approaches for mixture interpretation.

## ACKNOWLEDGEMENTS

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