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Y-STR Testing:
Enhancing Sexual Assault Workflows

> **The information in this brief applies to cold case sexual assaults as well as current case sexual assaults. Mentions of sexual assault apply to both types of sexual assault cases.**

Incorporating Y-STR testing (Y-chromosomal testing) into a sexual assault workflow can be a powerful tool for detecting male DNA foreign to the victim when traditional, autosomal short tandem repeat (STR) testing fails to aid the investigation. During traditional STR testing, male DNA may be masked or in competition with excess amounts of female DNA, which may result in partial or no male STR DNA results. Y-STR testing explicitly targets STR regions on the male Y chromosome that is passed down through the paternal lineage (i.e., father to son). By specifically targeting the Y-chromosome, a Y-STR profile can be unmasked in the presence of female DNA. Table 1 outlines several benefits for incorporating Y-STR testing in sexual assault workflows.

Table 1. Benefits of Y-STR Testing: Y-STR analysis can enhance DNA analysis workflows to help detect male DNA.

BENEFITS OF Y-STR TESTING
Target male-only DNA in mixed samples (i.e., samples having more than one source of DNA)
Determine number of male donors in a mixed sample
Resolve male-to-male mixtures
Provide clarity for inconclusive STR results
Aid in power of exclusion
Detect male DNA from cases involving ◆ azoospermic or vasectomized males, ◆ saliva following showering, ◆ digital penetration, ◆ no ejaculation, ◆ aged or improperly stored sexual assault kits in which sperm cells may be degraded, and ◆ extended time intervals between incident and collection.

Y-STR testing is more sensitive than common biological fluid screening methods, such as traditional serology techniques, and even some quantification methods that screen for total amounts of male and human DNA.[1] Thus, Y-STR profiles have been developed in cases in which seminal fluid or sperm were not detected by serology or when quantified male DNA is at a low level or even below the limit of detection. Y-STR analysis provides hope in reinvestigating sexual assault cases that may have gone cold, have screened negative, or have produced only the victim's DNA.

Y-STR Analysis: New Hope for Sexual Assault Cases

- Cold case reinvestigations
- Negative screenings
- Victim DNA only

Newer STR commercial kits—such as PowerPlex®Fusion, PowerPlex®Fusion 6C, AB GlobalFiler™, and QIAGEN Investigator® 24plex—have incorporated at least one additional male-specific marker to assist with the following:

- Detection of male DNA
- Determination of the number of contributors in a mixture
- Guidance in decision-making for proceeding with Y-STR testing

In one study, combining autosomal STR testing with Y-STR testing resolved 1 in 10 cases with previous inconclusive STR results, detected an increase in the number of male contributors in a mixed sample, and provided highly informative DNA profiles in an additional 21% of cases.[1] Approaches that combine match probabilities of STR and Y-STR profiles to increase the rarity of a match will prove beneficial in cases in which there is a Y-STR profile with limited STR profile data.[2,3]

Vaginal and anal swabs were collected from a 15-year-old female 48 hours after an alleged penile penetration incident. No spermatozoa were found, but a 16-allele Y-STR profile that matched the suspect was developed from the vaginal swab.[4]

As technology improves, resulting in increases in sensitivity, the detection of male DNA in sexual assaults is becoming more achievable at extended intervals between an incident and the collection of samples. Although producing DNA profiles within 48–72 hours post-coital is common, Y-STR profiles are pushing the limits of 144 hours (6 days).[5] Enhanced methods, such as post-polymerase chain reaction (PCR) purification and nested PCR, have been successful in a research setting, detecting Y-STR profiles from properly collected cervicovaginal samples 9 days post-coital.[6] As emerging technologies are implemented in crime laboratories, policies about collection times may allow for longer periods between assault, exam, and collection.

A commonly cited limitation to Y-STR testing is the lack of discrimination power because of its haploid nature and inheritance pattern.[7] Commercial kits that in the past could not distinguish between related males—and in some circumstances, even unrelated males—have reduced that limitation. Connecting patrilineal lines is helpful for establishing ancestry and in missing persons or mass disaster events; however, further distinction between relatives would aid more criminal investigations. For criminal forensic use, research into rapidly mutating Y-STRs has shown an increase in differentiation between unrelated and related males.[8] Newer Y-STR commercial kits, such as PowerPlex® Y23 and YFiler™ Plus, have incorporated rapidly mutating Y-STR locations to increase the usefulness of Y-STR analysis in forensic investigations.

Ultimately leading to the success of Y-STR workflows is establishing local or national Y-STR databases. Until databases are created, having possible suspect reference samples will be critical for the success of a Y-STR program. Currently, the Combined DNA Index System (CODIS) accepts Y-STR profiles for missing person–related indexes, but CODIS does not house a national, criminal Y-STR database.[9] In Austria, the National DNA Database expanded to include Y-STRs based on an in-house study that a sexual perpetrator was identified using Y-STRs in 38 of 239 sexual offenses.[10] In the first 40 cases uploaded to Austria's expanded database, a common Y-STR profile linked 3 rape cases together, identifying a perpetrator for all 3 crimes. In addition, a link between 2 additional rapes identified two perpetrators as father and son. Success will continue to improve with the utilization of Y-STR analysis and growing the database.

> *Thanks to cold case funds and the latest Y-STR technology, the Boston Police Department solved the rape and murder mystery surrounding Mary Sullivan—a victim of the so-called Boston Strangler—almost 50 years after her death.*[11]

Continued shifts in DNA platforms, such as the implementation of massively parallel sequencing, will allow for even more efficient, combined autosomal STR and Y-STR workflows. Until those shifts occur, laboratories should consider the efficacy of current technology and how implementing Y-STRs can improve solvability in sexual assaults cases.

References:

1. Purps, J., Geppert, M., Nagy, M., & Roewer, L. (2015). Validation of a combined autosomal/Y-chromosomal STR approach for analyzing typical biological stains in sexual-assault cases. *Forensic Science International: Genetics, 19*, 238–242. doi:10.1016/j.fsigen.2015.08.002
2. Walsh, B., Redd, A. J., & Hammer, M. F. (2008, January 30). Joint match probabilities for Y chromosomal and autosomal markers. *Forensic Science International, 174*, 234–238. doi:10.1016/j.forsciint.2007.03.014
3. Buckleton, J., & Myers, S. (2014, July 11). Combining autosomal and Y chromosome match probabilities using coalescent theory. *Forensic Science International: Genetics, 11*, 52–55. doi:10.1016/j.fsigen.2014.02.009
4. McDonald, A., Jones, E., Lewis, J., & O'Rourke, P. (2015, March). Y-STR analysis of digital and/or penile penetration cases with no detected spermatozoa. *Forensic Science International: Genetics, 15*, 84–89. doi:10.1016/j.fsigen.2014.10.015
5. Mayntz-Press, K. A., Sims, L. M., Hall, A., & Ballantyne, J. (2008, March). Y-STR profiling in extended intervals (≥ 3 days) postcoital cervicovaginal samples. *Journal of Forensic Science, 52*(2), 342–348. doi:10.1111/j.1556-4029.2008.00672.x
6. Hanson, E. K., & Ballantyne, J. (2014, October). A Y-short tandem repeat specific DNA enhancement strategy to aid in the analysis of late reported (≥ 6 days) sexual assault cases. *Medicine, Science and the Law, 54*(4), 209–218. doi:10.1177/0025802413519761
7. Roewer, L. (2009). Y chromosome STR typing in crime casework. *Forensic Science, Medicine, and Pathology, 5*, 77–84. doi:10.1007/s12024-009-9089-5
8. Ballantyne, K. N., Keerl, V., Wollstein, A., Choi, Y., Zuniga, S. B., Ralf, A., … Kayser, M. (2012, March). A new future of forensic Y-chromosome analysis: Rapidly mutating Y-STRs for differentiating male relatives and paternal lineages. *Forensic Science International: Genetics, 6*(2), 208–218. doi:10.1016/j.fsigen.2011.04.017
9. Federal Bureau of investigation (FBI) Laboratory. (2017, January 1). National DNA Index System (NDIS) Operational Procedures Manual, Version 5. Retrieved from https://www.fbi.gov/file-repository/ndis-procedures-manual-ver4-approved-04272016.pdf/view
10. Neuhuber, F., Klausriegler, E., Kreindl, G., Zahrer, W., Dunkelmann, B., Pickrahn, I., & Cemper-Kiesslich, J. (2013). The efficiency of Y-chromosome markers in forensic trace analysis and their inclusion in the Austrian National DNA Database. *Forensic Science International: Genetics, Supplement Series, 4*(1), e172–e173. doi:10.1016/j.fsigss.2013.10.089
11. Bulman, P. (2014, February). Solving Cases with DNA: The Boston Strangler Case. (NCJ244151). *NIJ Journal, 273*. Retrieved from https://www.nij.gov/journals/273/Pages/boston-strangler.aspx

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SAKI 10850 R2 0719

This project was supported by Grant No. 2015-AK-BX-K021 awarded by the Bureau of Justice Assistance. The Bureau of Justice Assistance is a component of the U.S. Department of Justice's Office of Justice Programs, which also includes the Bureau of Justice Statistics, the National Institute of Justice, the Office of Juvenile Justice and Delinquency Prevention, the Office for Victims of Crime, and the SMART Office. Points of view or opinions in this document are those of the author and do not necessarily represent the official position or policies of the U.S. Department of Justice. **For more information, visit www.sakitta.org.**