

Y-STR Testing:

Enhancing Sexual Assault and Cold Case Workflows

Incorporating Y-STR testing (Y-chromosomal testing) into a cold case 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 cold case 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 <ul style="list-style-type: none"> ◆ azoospermic or vasectomized males, ◆ saliva following showering, ◆ digital penetration, ◆ no ejaculation, ◆ aged or improperly stored sexual assault kits where 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 where 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 some hope in reinvestigating cases that

may have gone cold, have screened negative, or produced only the victim's DNA.

Y-STR Analysis: New Hope for Cold 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 where 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, known as 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 and cold cases.

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