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Use of Y-STRs for Cold Cases/Sexual Assaults

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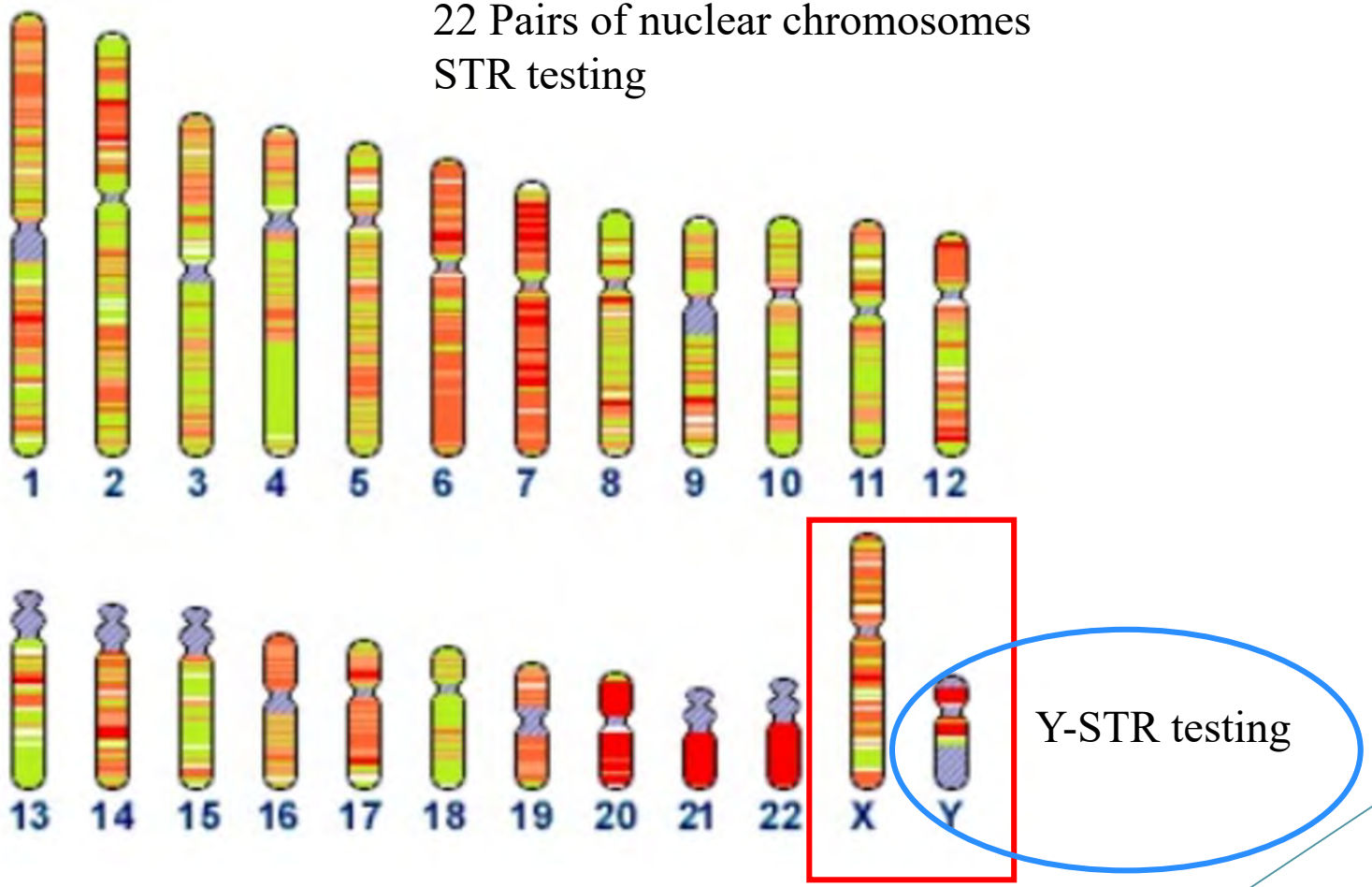
Outline

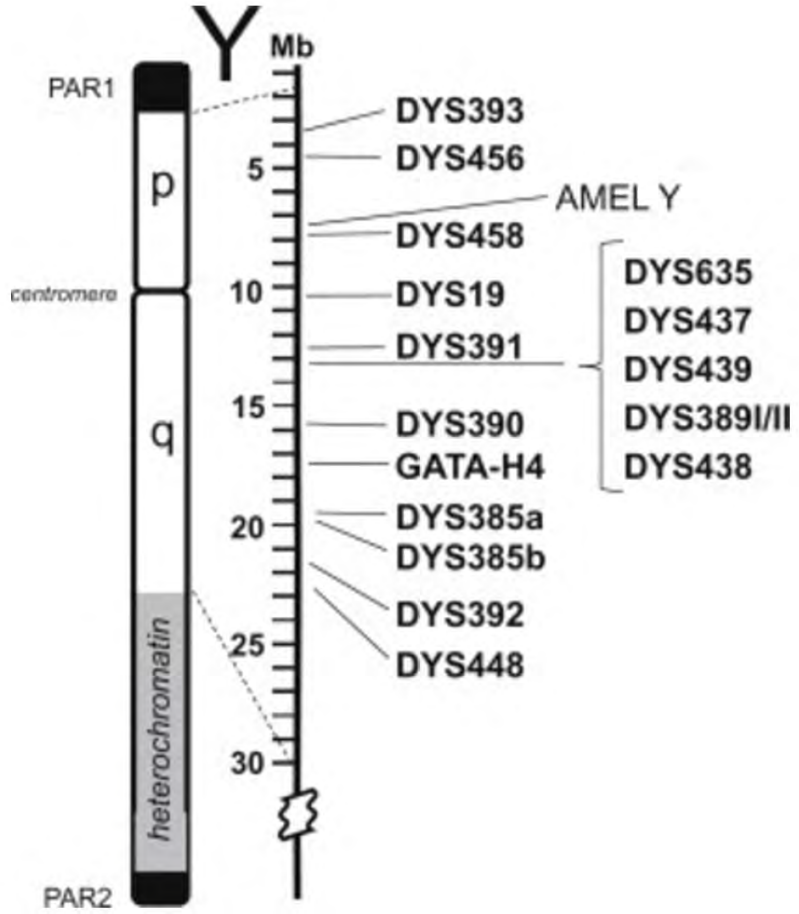
- Overview of Y-STR technology
- Applications of Y-STR testing
- Benefits and limitations of use
- Combined STR/Y-STR workflow approach
- Possible future advances

Human Chromosomes

22 Pairs of nuclear chromosomes
STR testing



Y-Chromosome



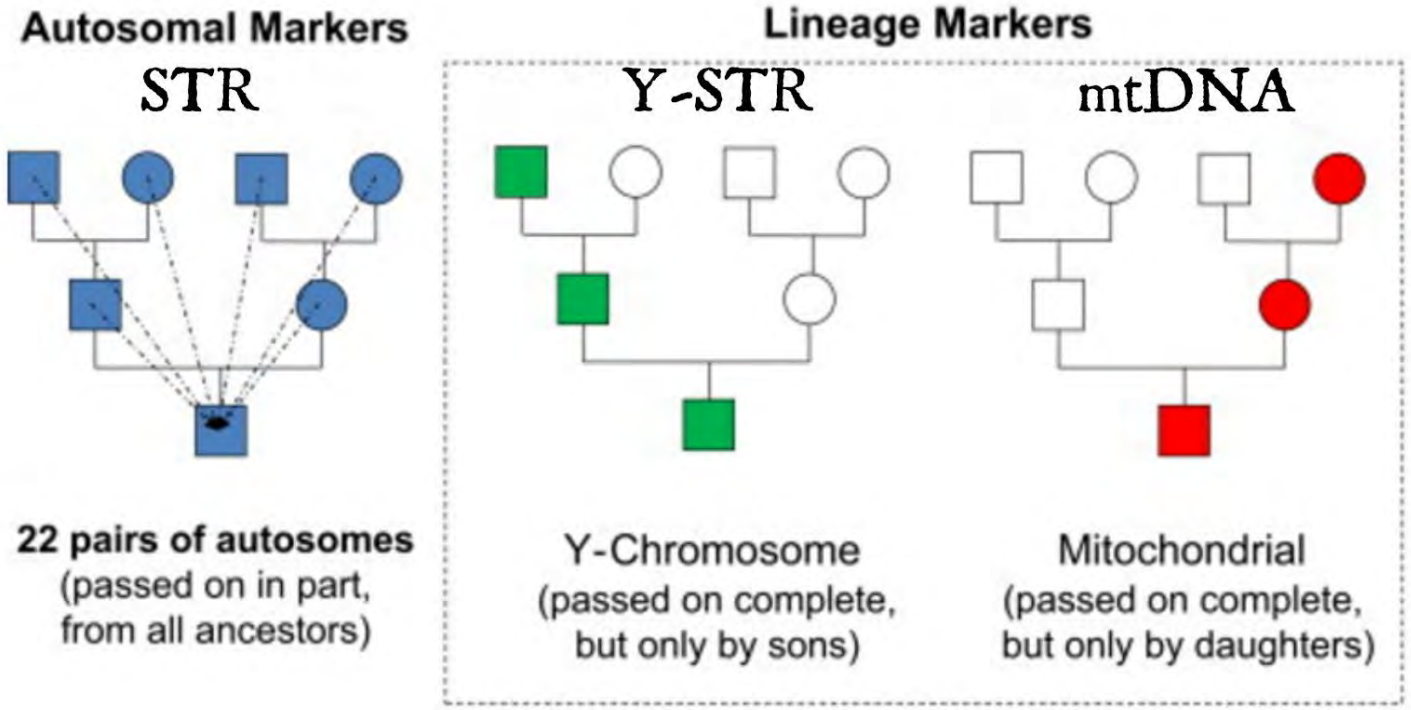
Y-STR Testing

-Targets specific locations on the Y-chromosome

- Common kits target 23-27 loci
- AMEL Y included in STR kits to test for possible sex determination
- Entire profile inherited together

John M. Butler, in *Advanced Topics in Forensic DNA Typing: Methodology*, 2012

Patterns of Inheritance



- Father and son will have the same Y-STR profile and Mother and her children will have the same mtDNA profile, barring any mutations
- Extend family references

John M. Butler, in *Advanced Topics in Forensic DNA Typing: Interpretation*, 2015

Y-Chromosome Lineage Markers

Commonly applied uses takes advantage of inheritance patterns

- Missing persons investigations
- Disaster victim identification
- Familial searching
- Kinship
- Genealogy
- Evolution

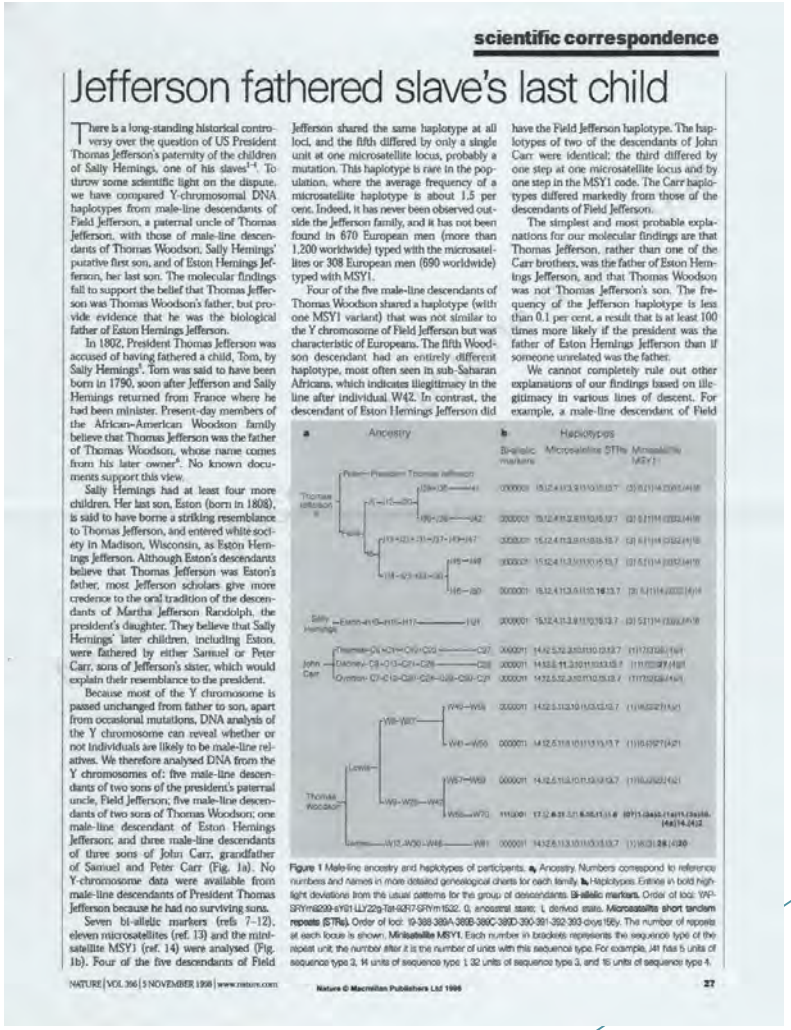
- Forensic Casework

Basic Limitations

- Inheritance of individual alleles is not independent
- Lower discrimination compared to STR testing

Y-STRs & Historical Questions

Was Thomas Jefferson the father of any of the children of his slave, Sally Hemings?



scientific correspondence

Jefferson fathered slave's last child

There is a long-standing historical controversy over the question of US President Thomas Jefferson's paternity of the children of Sally Hemings, one of his slaves[1–4]. To throw some scientific light on the dispute, we have compared Y-chromosomal DNA haplotypes from male-line descendants of Field Jefferson, a paternal uncle of Thomas Jefferson, with those of male-line descendants of Thomas Woodson, Sally Hemings' putative first son, and of Eston Hemings Jefferson, her last son. The molecular findings fail to support the belief that Thomas Jefferson was Thomas Woodson's father, but provide evidence that he was the biological father of Eston Hemings Jefferson.

In 1802, President Thomas Jefferson was accused of having fathered a child, Tom, by Sally Hemings[5]. Tom was said to have been born in 1790, soon after Jefferson and Sally Hemings returned from France where he had been minister. Present-day members of the African-American Woodson family believe that Thomas Jefferson was the father of Thomas Woodson, whose name comes from his later owner[6]. No known documents support this view.

Sally Hemings had at least four more children. Her last son, Eston (born in 1808), is said to have borne a striking resemblance to Thomas Jefferson, and entered white society in Madison, Wisconsin, as Eston Hemings Jefferson. Although Eston's descendants believe that Thomas Jefferson was Eston's father, most Jefferson scholars give more credence to the oral tradition of the descendants of Martha Jefferson Randolph, the president's daughter. They believe that Sally Hemings' later children, including Eston, were fathered by either Samuel or Peter Carr, sons of Jefferson's sister, which would explain their resemblance to the president.

Because most of the Y chromosome is passed unchanged from father to son, apart from occasional mutations, DNA analysis of the Y chromosome can reveal whether or not individuals are likely to be male-line relatives. We therefore analysed DNA from the Y chromosomes of: five male-line descendants of two sons of the president's paternal uncle, Field Jefferson; five male-line descendants of two sons of Thomas Woodson; one male-line descendant of Eston Hemings Jefferson; and three male-line descendants of three sons of John Carr, grandfather of Samuel and Peter Carr (Fig. 1a). No Y-chromosome data were available from male-line descendants of President Thomas Jefferson because he had no surviving sons.

Seven bi-allelic markers (refs 7–12), eleven microsatellites (ref. 13) and the mini-satellite MSY1 (ref. 14) were analysed (Fig. 1b). Four of the five descendants of Field Jefferson shared the same haplotype at all loci, and the fifth differed by only a single unit at one microsatellite locus, probably a mutation. This haplotype is rare in the population, where the average frequency of a microsatellite haplotype is about 1.5 per cent. Indeed, it has never been observed outside the Jefferson family, and it has not been found in 670 European men (more than 1,200 worldwide) typed with the microsatellites or 308 European men (690 worldwide) typed with MSY1.

Four of the five male-line descendants of Thomas Woodson shared a haplotype (with one MSY1 variant) that was not similar to the Y chromosome of Field Jefferson but was characteristic of Europeans. The fifth Woodson descendant had an entirely different haplotype, most often seen in sub-Saharan Africans, which indicates illegitimacy in the line after individual W4Z. In contrast, the descendant of Eston Hemings Jefferson did have the Field Jefferson haplotype. The haplotypes of two of the descendants of John Carr were identical; the third differed by one step at one microsatellite locus and by one step in the MSY1 code. The Carr haplotypes differed markedly from those of the descendants of Field Jefferson.

The simplest and most probable explanations for our molecular findings are that Thomas Jefferson, rather than one of the Carr brothers, was the father of Eston Hemings Jefferson, and that Thomas Woodson was not Thomas Jefferson's son. The frequency of the Jefferson haplotype is less than 0.1 per cent, a result that is at least 100 times more likely if the president was the father of Eston Hemings Jefferson than if someone unrelated was the father.

We cannot completely rule out other explanations of our findings based on illegitimacy in various lines of descent. For example, a male-line descendant of Field

Figure 1 Male-line ancestry and haplotypes of participants. a, Ancestry. Numbers correspond to reference numbers and names in more detailed genealogical charts for each family. b, Haplotypes. Entries in bold highlight deviations from the usual patterns for this group of descendants. **Bi-allelic markers.** Order of loci: YAP-SRY=8299-sY81-LLY22g-Tat-92R7-SRY=1532. 0, ancestral state; 1, derived state. **Microsatellite short tandem repeats (STRs).** Order of loci: 19-388-389A-389B-389C-389D-390-391-392-393-dye 156y. The number of repeats at each locus is shown. **Minisatellite MSY1.** Each number in brackets represents the sequence type of the repeat unit; the number after it is the number of units with this sequence type. For example, [4] has 5 units of sequence type 3, 14 units of sequence type 1, 32 units of sequence type 3, and 16 units of sequence type 4.

Y-STR & Missing Persons

1985 Covington, VA

- Two teenagers were at a gas station when it was firebombed
  - One of the teenagers was later arrested
  - The other one (Michael Purdue) likely drowned trying to swim across the Jackson River in Virginia
- Two fisherman later recovered remains on the riverbank believed to be Michael
- The family could not be located and the case went unsolved

Y-STR & Missing Persons

2016 Case Revisited

- 3D image created of what the missing person may have looked like but it did not provide leads

- A Y-STR profile was developed from the remains
- Authorities searched for paternal relatives of Michael
- Michael's brother Wally Purdue was located and provided a DNA sample

Y-STR & Missing Persons

- Wally Purdue could not be excluded as a paternal relative of the human remains recovered from the riverbank
- Human remains identified as belonging to Michael Purdue

Forensic Casework

- Y-chromosome explored forensically ~2001
- By 2003/2004 availability of commercial kits and **court acceptance**
- Early testing looked at 6 loci, popularized with 12-17 loci
- Current **commercial kits** examine 23-27 loci
- Creation/updates of Y-chromosome **population databases**

    example: US Y-STR Database

Forensic Casework

Main Applications

- Traditional, autosomal short tandem repeat (STR) testing fails to aid an investigation
- Male DNA is masked or in competition with excess amounts of female DNA
- Target Y-chromosome to develop a Y-STR DNA profile in the presence of female DNA

Forensic Casework

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

Forensic Casework

Detect male DNA from cases involving

- 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.

Forensic Casework

Y-STR Analysis: New Hope for Cold Cases

- Cold case reinvestigations
- Negative screenings
- Victim DNA only

Y-STR testing is more sensitive than common biological screening methods and even some quantification methods

Forensic Casework

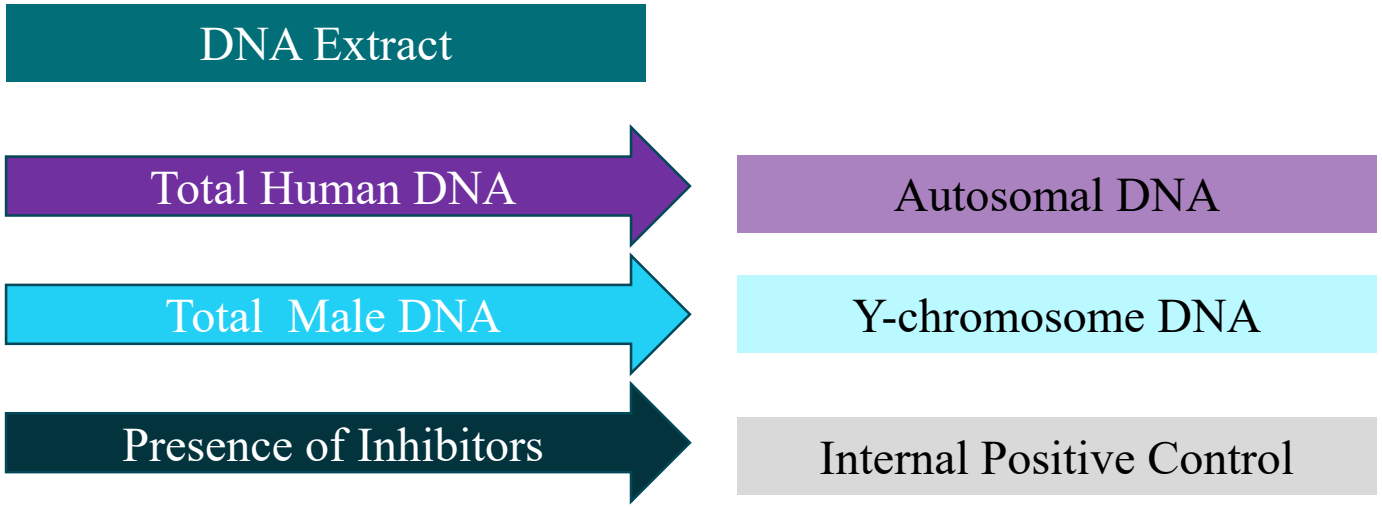
Vaginal and anal swabs were collected from a 15-year-old female 48 hours after an alleged penile penetration incident. No spermatozoa were found, but a 16-allele Y-STR profile that matched the suspect was developed from the vaginal swab.[4]

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

Data driven decision making

Human/Male DNA Quant -Aids in Decision Making

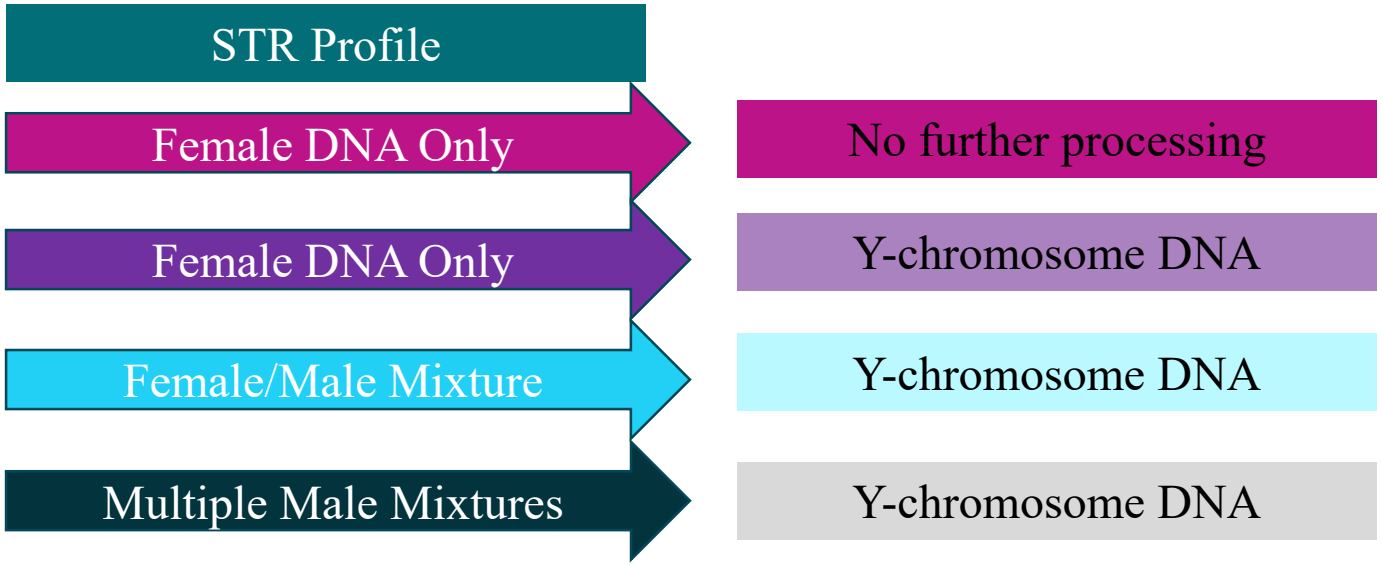
- Determine if enough DNA to proceed with downstream processing
- Evaluate mixture ratios to determine if enough male DNA to proceed with STRs or YSTRs
- Evaluates quality of DNA extraction



Data driven decision making

STR Profile Results-Aids in Decision Making

- STR data along with quantification data can help determine Y-STR analysis and aid in developing a male profile or even resolving male mixtures



Alternative approach- Combined STR/Y-STR

Extract	Amplify	Analyze	Review	Report
Remove possible DNA from substrate	DNA is copied by targeting STR & Y-STR regions	STR Regions are separated and with aid of computer software DNA profile is generated	100% of DNA exams undergo technical and administrative review	Results, comparisons, conclusions, and statistics are summarized in a forensic case report

Combined STR/Y-STR Workflow

Y-STR testing detected multiple male contributors in biological samples approximately three times more often than with autosomal STR testing

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

Combining autosomal STR testing with Y-STR testing resolved:

- 1 in 10 cases with previous inconclusive STR results
- Provided highly informative DNA profiles in an additional 21% of cases

Combined STR/Y-STR Workflow

- Produce results from various body orifices and touch samples
- Resolve complex mixtures that are inconclusive for STRs
- Increased chance of detecting male donors
- Help individualize males in mixtures
- Ensures retrieval of the maximum amount of information
- Provide additional leads for investigation and prosecution
- Improves match rarity

Technology Advances-
Extended Time Intervals Between Assault and Collection

Typical time intervals to produce a DNA profile:

48-72 hours post-coital

Y-STR profiles are pushing the limits of:

144+ hours (6 days)

Enhanced methods:

- Post-amplification purification
- Nested PCR

Commercial kit (Yfiler Plus 27 loci):

- 4 days- 32%
- 7 days- 7%
- 9 days- 11%

Ballantyne J., Hanson E., Green R., Holt A., Mulero J.. (2013). Enhancing the sexual assault workflow: Testing of next generation DNA assessment and Y-STR systems. *Forensic Science International: Genetics ,Supplemental Series 4(1)*, 228-229. http://doi.org/10.1016/j.fsigss.2013.10.117

Technology Advances-
Rapidly Mutating Y-STRs

- Connecting patrilineal lines is helpful for establishing ancestry and in missing persons or mass disaster events

Research/selection of Y-STR markers with high mutation rates can help with differentiation between unrelated and related males

- Patrilineal inheritance can be a hindrance to distinction between male relatives

Newer commercial kits have incorporated RM Y-STRs to help with forensic casework

Technology Advances-
Massively parallel sequencing

Shift in DNA platform allows simultaneous analysis of STRs and Y-STRs

- One test can provide information from over 200 locations
- Target subsets of the human genome that are forensically relevant
- Improved analysis of degraded samples

Case Example- Mary Sullivan

Jan 4th 1964

- Found dead in her home she shared with roommates
- Nylon stocking around her face, scarves tied around her neck
- Believed to be raped and strangled; the 13th victim of the Boston Strangler
- Albert DeSalvo confessed to this crime but later recanted before his death in 1973

Case Example- Mary Sullivan

July 2013

- Slides taken from Sullivan's body and a blanket with seminal fluid from the murder site was matched to DeSalvo's nephew via Y-STR testing
- DeSalvo's remains were then excavated in order to obtain a direct reference sample
- The direct confirmation sample confirmed DeSalvo raped and murdered Mary Sullivan

Y-STR Analysis-
Requires Reference Samples

Mass Disaster: Victim remains are compared to living relatives

Kinship: child/missing person compared to alleged relatives

Genealogy: Compare Y-STR results between relatives

Forensic Casework: Need direct suspect reference for confirmation

Y-STR Analysis-
Requires Reference Samples

Y-STR profiles for forensic casework are not currently stored in the US Database- CODIS

Forensic casework Y-STR profiles cannot be searched in CODIS

Reference samples are **critical**

for the success of a Y-STR program

Database Example- Austria expands National DB to include Y-STRs

- A sexual perpetrator was identified using Y-STRs in 38 of 239 sexual offenses
- In the first 40 cases to upload
  - 3 rape cases were linked
  - 2 other rapes identified perpetrators as father and son

Success will continue to improve with utilization of Y-STR analysis and growing the database

Summary

- Y-STR testing occurs on the Y-chromosome that is only found in males
- The Y-chromosome is paternally inherited making it a lineage marker
- Inheritance patterns make Y-STR testing very useful in missing persons, familial searching, & kinship

- Applications in forensic casework include:
  - Cold case investigations
  - Cases that screened negative
  - STR testing inconclusive
  - High levels of female DNA mask male DNA
  - Resolve number of males in a sample

Summary

- Data driven decision making- usefulness of Y-STRs can be evaluated:
  - During quantification
  - Following STR profiling
  - If references are available
- Advances in technology
  - Enhanced Y-STR kits are making it possible to obtain Y-STRs profiles 6-9 days post-coital
  - Rapidly mutating Y-STRs helping to aid in criminal casework to distinguish between male relatives
  - Changes in DNA platforms will make it easier to type STRs and Y-STRs at the same time