Extending the Time to Collect DNA in Sexual Assault Cases

by Terry Taylor

New research offers hope for extending the timeline for collecting samples suitable for DNA profiling in sexual assault cases.

DNA profiling plays a major role in sexual assault cases, but it has a significant shortcoming: Using the current standard DNA profiling method, many jurisdictions require the collection of evidentiary samples within three days of the assault. After that, it may become difficult to obtain a usable DNA profile of the male suspect.

What if we could extend the window of time for collecting evidence?

We know that sperm cells are found in the female reproductive tract for seven days after ejaculation or longer. Researchers are testing a hypothesis that may extend the length of time in which DNA profiling is possible in sexual assault cases.

Dr. Jack Ballantyne, professor of chemistry at the University of Central Florida in Orlando and an internationally recognized expert on DNA profiling, believes that real evidence is being lost because of some jurisdictions' adherence to the three-day window.[1] Ballantyne believes that new techniques may make it possible to collect samples for as long as five to six days after a sexual assault and still do viable analysis.

A DNA profile is a set of numbers that represent regions, or loci, of variable, repeated DNA sequences. Short sequences of DNA that repeat a number of times are known as short tandem repeats (STRs). The number of times a sequence repeats varies from person to person and,

except in the case of identical twins, this variability can be used to tell people apart. STR analysis is a forensic technique that develops and distinguishes DNA profiles by evaluating these regions.

Most DNA is located in the nucleus of the cell. Within the nucleus, DNA is divided into long, tightly coiled pieces called chromosomes. Humans have 23 pairs of chromosomes. Of these, 22 pairs are autosomal chromosomes (or autosomes), which are not involved in determining a person's sex. The other pair are sex-determining chromosomes. There are two types of sex chromosomes – X chromosomes and Y chromosomes. Women have two X chromosomes; men have one X chromosome and one Y chromosome. (See Fig. 1, page 25.)

STR analysis is traditionally performed on autosomes because the manner in which they are inherited results in a higher degree of variation than Y chromosomes and thus produces profiles that have far stronger statistical power.[2] (See sidebar, "STR Analysis.")

The standard DNA profiling technique of using STR analysis, however, has shortcomings. One, as mentioned above, is that it is difficult to obtain a DNA profile using STR analysis more than three days after a sexual assault.

A second shortcoming of current DNA profiling techniques is the inability to get a male profile when — in evidence collected after an alleged sexual assault — female cells vastly outnumber the available sperm cells. In such cases, the amount of the female's DNA is so great compared to the male's DNA that the female DNA swamps the process and laboratory analysis is extremely difficult. (See sidebar, "New Technologies Promise Better Future Results.")

An approach that has shown promise in solving both of these difficulties is called Y-STR analysis, based on the male Y chromosome. An NIJ-funded pilot study of Y-STR DNA profiling by Ballantyne and his colleagues offered hope for a solution to the problem of analyzing postcoital samples collected more than three days after the incident.[3] His team was able to obtain complete Y-STR profiles on samples collected as many as five days after coitus and a partial profile at six days.

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Y-STR analysis also helps solve the swamping problem because it is selective for Y chromosome DNA, which has little overlap with female DNA. As long as the quantity of male DNA is sufficient, the amount of female DNA present is not a problem.

Ballantyne is quick to caution that Y-STR profiling "cannot supplant autosomal DNA profiling." Y-STR profiles do not have the statistical power of autosomal STR profiles and therefore do not carry the same power to discriminate among individuals. In many cases, scientists are unable to obtain a DNA profile based on Y chromosome DNA, and only a few Y-STR systems are currently available for use.[4]

The likelihood of obtaining an autosomal DNA profile, however, is small in several situations:

- When a sample contains both male and female body fluids other than semen (e.g., saliva-saliva, saliva-vaginal fluids, fingernail scrapings).
- When the number of sperm is low, or they are in a fragile state.
- When there is more than one semen donor, as in cases of multiple perpetrator rape.

Ballantyne and his colleagues continue their work to increase the number of Y-STR systems, but other than adding more Y-STR loci to the analysis, little can be done to change Y chromosome DNA's relative lack of power to distinguish individuals — the greatest weakness of this type of DNA profiling.

Still, Y-STR profiling is sometimes the only option available.[5]

Because their pilot study involved collection of postcoital samples from only three consenting male-female couples, Ballantyne and his team

STR Analysis

The most common type of DNA profiling today for criminal cases and other types of forensic uses is called "STR" (short tandem repeat) analysis.

Using DNA to distinguish between two individuals is a tricky matter, because close to 99.9 percent of our DNA is the same as everybody else's DNA.[1] DNA that actually codes for proteins cannot vary much without rendering the proteins ineffective. The four nucleotide bases that make up the backbone of DNA provide instructions for assembling the amino acids in proteins by being in a precise sequence, with each three-base group coding for a specific amino acid. If that DNA base sequence is altered (or "mutated," as scientists generally say), the sequence of amino acids in the resulting protein can also be altered. As a result, because protein function derives from a specific amino acid sequence, the protein may not work.

Think of DNA as the "blueprint" for a house and proteins as the steel, timber, bricks and mortar, from which the house will be built. A brick that is mostly sand instead of clay will crumble, and mortar with the wrong ratio of cement to aggregate will fail. Likewise, a protein with the wrong sequence of amino acids often won't function. (This analogy fails to capture the complexity of the DNA-protein system, however, because proteins are not only the "bricks" and "timber." Some "read" the "blueprint" and supervise the building, others are the "bricklayers" and "carpenters," and still others maintain and keep the house functioning after it is built.) Non-functional or missing proteins are the basis for many genetic diseases. Useful differences in the DNA must be found in the remaining one-tenth of one percent, which is not known to code for anything specific. Because this section of the DNA's precise sequence is not so important, it is quite variable, which makes it possible to use DNA to distinguish between individuals.

Among the 3 million or so DNA bases that do not code for proteins are regions with multiple copies of short repeating sequences of these bases, which make up the DNA backbone (for example, TATT).* These sequences repeat a variable number of times in different individuals. Such regions are called "variable-number short tandem repeats," and they are the basis of STR analysis. A collection of these can give nearly irrefutable evidence statistically of a person's identity because the likelihood of two unrelated people having the same number of repeated sequences in these regions becomes increasingly small as more regions are analyzed.

Autosomal chromosomes are those not involved in determining a person's gender, and STRs on these chromosomes are called autosomal STRs. Other STRs used for forensic purposes are called Y-STRs, which are derived solely from the male sex-determining Y chromosome. Profiles based on autosomal STRs provide far stronger statistical power than profiles based on Y-STRs, because autosomal DNA is randomly exchanged between matched pairs of chromosomes in the process of making egg and sperm cells. That's how, with billions of humans on the planet, no two people who are not identical twins are exactly alike. Profiles based on Y-STRs are statistically weaker because only males have a Y chromosome and all males get theirs from their fathers, so all males in any paternal line have nearly identical Y chromosomes. Given enough Y-STRs, which scientists call loci, a Y-STR profile can offer substantial power to discriminate between individuals, but this type of profile is certainly not as powerful as an autosomal STR profile.

plan to add statistical power to their experimental evidence with a new NIJ-funded study that will include 150 consenting male-female couples. His co-principal investigator is Dr. Patricia M. Speck, associate professor of nursing at the University of Tennessee Health Science Center College of Nursing in Memphis.

"Given the variables in the collection of samples, we don't know what we can do and with whom," Speck explained. The large study aims to expand knowledge about the limits of DNA profiling in sexual assault cases. The study will examine the impact of a number of factors

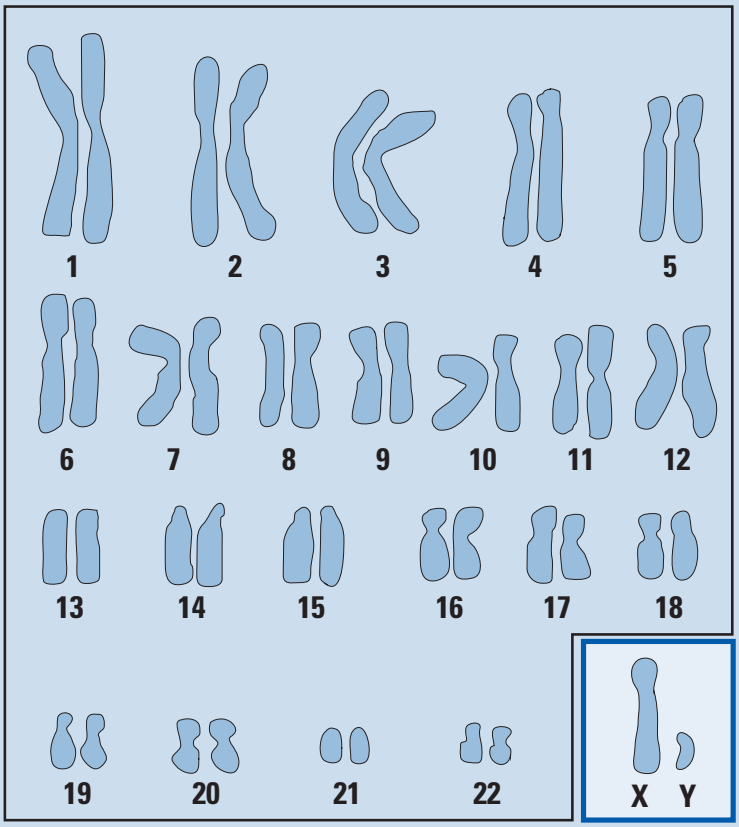
In the United States, 13 autosomal STR loci are now accepted as the system used for forensic purposes.[2] Given a robust crime scene DNA sample with good data for all 13 STRs, the likelihood of a person unrelated to the actual perpetrator having a perfect match for all 13 is typically around 1 in 1 billion. By contrast, experimental work with a very robust set of 30 Y-STR loci showed a probability of about 1 in 50,000 for a perfect match.[3]

* TATT stands for a specific string of nucleotide bases, thymine-adenine-thymine-thymine. Thymine and adenine are two of the four bases frequently found in DNA. The other two are cytosine (C) and guanine (G).

Notes

1. Basics of DNA Typing. http://dna.gov/basics/ (accessed July 7, 2010).
2. Norrgard, K., "Forensics, DNA Fingerprinting, and CODIS," *Nature Education* 1(1) (2008): http://www.nature.com/scitable/topicpage/forensics-dna-fingerprinting-and-codis-736.
3. Hanson, E., and J. Ballantyne, "A Highly Discriminating 21 Locus Y-STR 'Megaplex' System Designed to Augment the Minimal Haplotype Loci for Forensic Casework," *Journal of Forensic Sciences* 49 (January 2004): 1-12.

Figure 1. Human Chromosomes



Humans have 23 pairs of chromosomes – 22 pairs of autosomes and one pair of chromosomes that determine gender (the X and Y chromosomes).

— from sampling timeline to menstruation cycle — on the ability of investigators to obtain a DNA profile. To ensure rigorous, uniform methods, qualified forensic nurses will collect all samples. After the couples have abstained from sexual intercourse for 10 days, they will engage in unprotected intercourse, and vaginal samples will be collected at 4, 7 and 9 days, with each collection following a new unprotected coitus episode. The collection process will also include a cervical sample because sperm cells live longer in the cervix than in the vagina, where they are quickly eliminated.

New Technologies Promise Better Future Results

A routine difficulty encountered in producing autosomal DNA profiles in rape cases is obtaining enough sperm cells for the analysis. Cells can be damaged or degraded due to time, or simply stuck to epithelial cells (those that line the cavities and structures of the body, such as the vagina), making it difficult to carry out a standard autosomal STR analysis.

A session at the 2010 NIJ Annual Conference highlighted two promising technologies for enhancing the separation of sperm cells from other cells in a mixed sample. Gary Stacey, vice president of technology at Haemonetics Corporation, presented a technique called holographic optical trapping (HOT). HOT is an automated system now commercially available from Arryx, a Haemonetics subsidiary. This system allows small particles, such as sperm cells or cell fragments, to be directly manipulated using a computer-controlled hologram array. Stacey showed video and presented data offering evidence that HOT can separate sperm cells from female epithelial cells (or other contaminants) before micro-dissection to obtain DNA, enhancing the resulting STR analyses. The system is simple to use, provides visual confirmation of the process as it proceeds and allows video data tracking.

Henry K. Lin, a member of the Biosciences Division, of the Oak Ridge National Laboratory, discussed a microfabricated filter technology originally developed to separate metastatic cancer cells from the blood. Because sperm cells are substantially smaller than the female epithelial cells with which they are likely to be mixed in rape cases, this two-tier filtration process first selects out the larger cells, allowing the sperm cells to pass through and be drawn off separately. Experiments testing this application of the system have successfully separated sperm cells in mixtures where they were outnumbered 25 to 1, with a higher DNA recovery rate and cleaner STR profiles than are obtained with standard methods. The investigators intend to continue this research with epithelial cell-to-sperm cell ratios up to 100 to 1.

Ballantyne explained the importance of such research: "If we can separate sperm cells accurately and efficiently in challenging samples, it will permit standard autosomal DNA analysis in more cases. This will be a great move forward."

Participants in the study will also be asked questions about their menstrual cycle, general health background and various social indicators. To help answer questions about the effects of menstruation on DNA collection, participants will be divided between women whose sample collection comes before menstruation and women whose menstruation has started between the episode of intercourse and the sample collection.

"The variables are complex and potentially confounding," Speck said. An open question is whether estrogen protects from injury. "Knowing this could be important to testimony about injury in a sexual assault case

because where the woman is in her menstrual cycle at the time of the event could play a role in whether she is physically injured."

According to Ballantyne, another possible aim of the study will be to document exactly when autosomal DNA analysis fails. At seven or nine days, the current consensus is that there is no hope for an autosomal STR profile. "If we successfully obtain a Y-STR profile with a sample collected seven or nine days later, we can perhaps use the expertise we have gained to also tease out an autosomal profile," Ballantyne explains. "We'll be more certain whether or not it should be a routine matter to collect samples more than a few days after the assault. For example, if the study shows that more than 50 percent of the samples yield a reportable DNA profile after four days, it would have important policy implications. It won't be just a report in a scientific journal."

Speck agrees: "This study could have the effect of changing the timeframe for bringing victims in, or it could confirm cutting off sample collection earlier." The important thing is to develop interventions that will help victims. "The bottom line is," she said, "we want to take rapists off the street."

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Notes

1. The three-day window may be in part due to the jurisdiction's protocol, the laboratory's protocol or both.
2. For each of their 22 pairs of autosomes, children inherit one autosomal chromosome from each parent. The autosomes the child receives, however, are not identical to their parents' autosomes — before a parent passes on his or her autosomes, each pair goes through a process called recombination, which randomly shuffles that pair. The mixed autosome produced by that process is what gets passed on to the child from the parent. In the mother, the X chromosome pair also goes through recombination, just like autosomes, and the mixed X chromosome is passed on to the son or daughter. Because men only have one X chromosome, recombination does not occur and they pass their X chromosome on unchanged to their daughters. Similarly, because men only have one Y chromosome, it is passed unchanged to their sons.
3. Mayntz-Press, K., L. Sims, A. Hall, and J. Ballantyne, "Y-STR Profiling in Extended Interval (≥ 3 Days) Postcoital Cervicovaginal Samples," *Journal of Forensic Sciences* 53 (March 2008): 342-348.
4. A collection of Y-STR markers or Y-STR loci (see sidebar, "STR Analysis") must be discovered and validated as useful before it can be used as a Y-STR system for DNA profiling.
5. In addition to its usefulness in sexual assault cases, investigators might be able to identify a disaster victim using the Y-STR profile of a close male relative.