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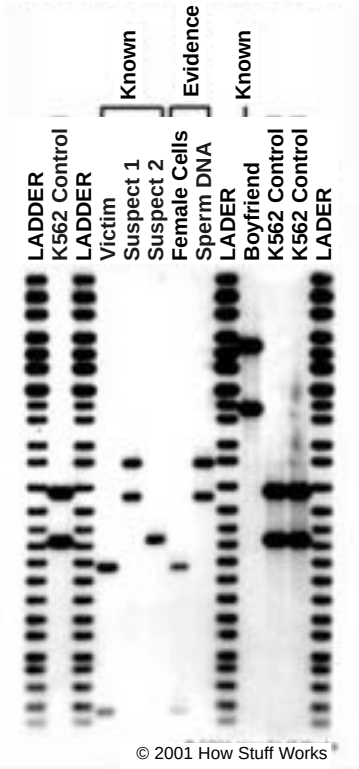
How DNA Evidence Works

by An Meeker-O'Connell

The public has always been captivated by the drama that occurs in the courtroom. There is even a whole channel, CourtTV, devoted to showing real court cases as they wend their way through the legal system. TV shows and movies depict passionate attorneys sparring verbally as they fight to convict or acquit the accused. However, the most tense moments of a criminal trial are likely those that go unseen: the jury deliberations.

Sexual Assault Case



After both sides present their evidence and argue their cases, a panel of jurors must weigh what they have heard and decide whether or not the accused person is guilty as charged. This can be difficult. The evidence presented is not always clear-cut, and sometimes jurors must decide based on what a witness says they saw or heard. Physical evidence can be limited to strands of hair or pieces of fabric that the prosecution must somehow link conclusively to the defendant.

What if there was a way of tying a person to the scene of a crime, beyond a shadow of a doubt? Or, more importantly, what if you could rule out suspects and prevent the wrong person from being locked up in jail? This dream is beginning to be realized through the use of DNA evidence. In this issue of How Stuff Works, we'll look at how DNA fingerprinting works, and what DNA evidence can be used for.

The Science of DNA Fingerprinting

Proving that a suspect's DNA matches a sample left at the scene of a crime requires two things:

- Creating a DNA profile using basic molecular biology protocols.
- Crunching numbers and applying the principles of population genetics to prove a match mathematically.

Your Own Personal Barcode?

We all like to think that we are unique, not like anyone else in the world. Unless you are an identical twin, at the nuclear level, you are! Humans have 23 pairs of chromosomes containing the DNA blueprint that encodes all the materials needed to make up your body as well as the instructions for how to run it. One member of each chromosomal pair comes from your mother, and the other is contributed by your father.

Every cell in your body contains a copy of this DNA. While the majority of DNA doesn't differ from human to human, some 3 million base pairs of DNA (about 0.10 percent of your entire genome) vary from person to person. The key to DNA evidence lies in comparing the DNA left at the scene of a crime with a suspect's DNA in these chromosomal regions that do differ.

There are two kinds of polymorphic regions (areas where there is a lot of diversity) in the genome:

- Sequence polymorphisms
- Length polymorphisms

Sequence Polymorphisms
Sequence polymorphisms are usually simple substitutions of one or two bases in the genes themselves. Genes are the pieces of the chromosome that actually serve as templates for the production of proteins. Amazingly, despite our complexity, genes make up only 5 percent of the human genome. Individual variations within genes aren't very useful for DNA fingerprinting in criminal cases.

Non-coding DNA
The other 95 percent of your genetic makeup doesn't code for any protein. Because of this, these non-coding sequences used to be called "junk DNA," but it turns out that these regions do actually have important functions such as:

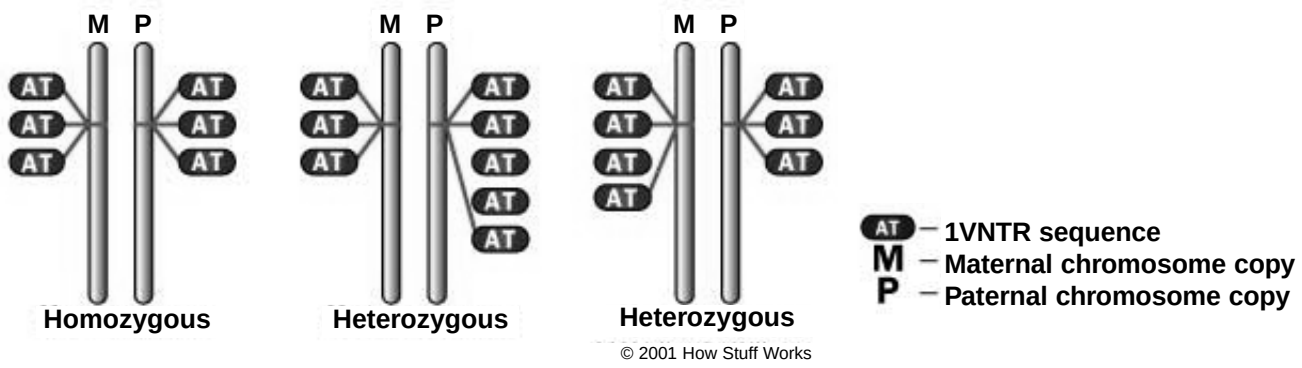
- Regulation of gene expression during development.
- Aiding or impeding cellular machinery from reading nearby genes and making protein.
- Serving as the bricks and mortar of chromosomal structure.

Length Polymorphisms
Non-coding DNA is full of length polymorphisms. Length polymorphisms are simply variations in the physical length of the DNA molecule.

DNA evidence uses a special kind of length polymorphism found in non-coding regions. These special variations come from stretches of short, identical repeat sequences of DNA. A particular sequence can be repeated anywhere from one to 30 times in a row, and so these regions are called variable number tandem repeats (VNTRs).

The size of a DNA fragment will be longer or shorter, depending on how many copies of a VNTR there are. In the case of DNA evidence, the great thing is that the number of tandem repeats at specific places (called loci) on your chromosomes varies between individuals. For any given VNTR loci in your DNA, you will have a certain number of repeats.

You inherit one copy of each chromosome from your mother and father. This means that you have two copies of each VNTR locus, just like you have two copies of real genes. If you have the same number of sequence repeats at a particular VNTR site, you are called homozygous at that site; if you have a different number of repeats, you are said to be heterozygous.



Creating a DNA profile: The Basics

The basic procedure used to isolate an individual's DNA fingerprint is called Restriction Fragment Length Polymorphism (RFLP) analysis. This is a complicated way of saying that investigators determine the number of VNTR repeats at a number of distinctive loci to come up with an individual's DNA profile.

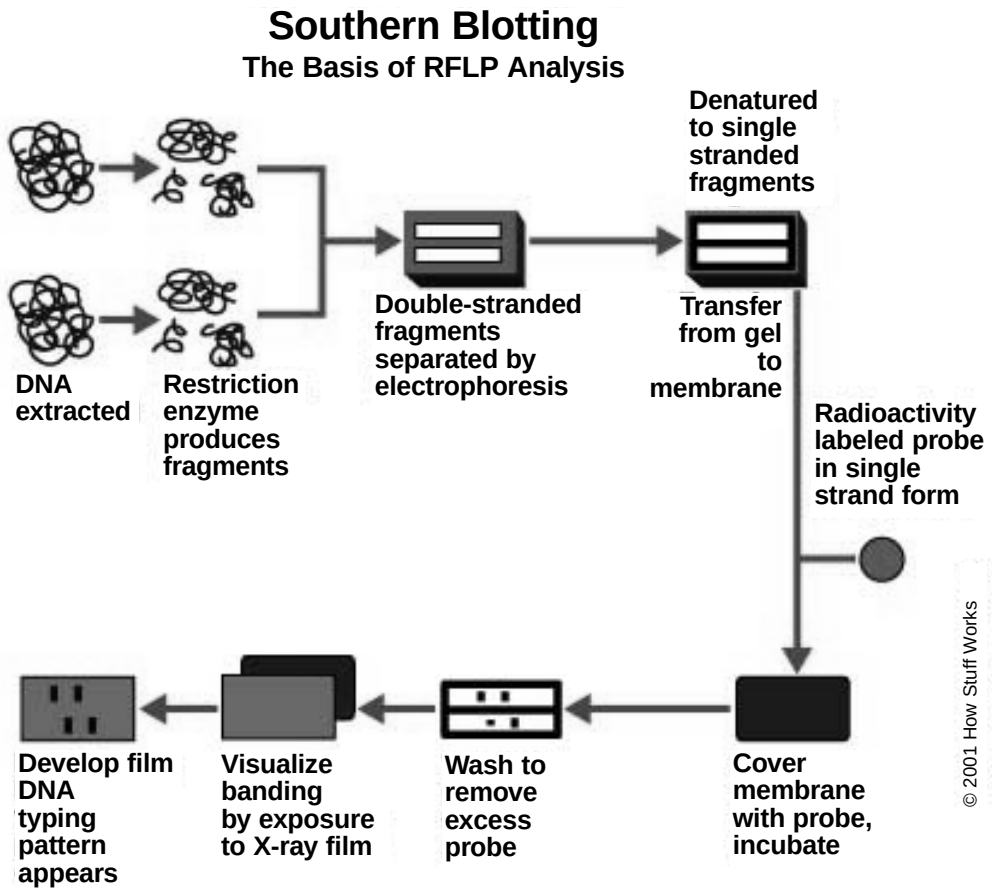
Here is the key to DNA evidence:

If you are looking at a particular person's DNA, and a particular VNTR area in that person's DNA, there is going to be a certain number of repeats in that area. What you do to make a DNA fingerprint is to count the number of repeats for a specific person for a specific VNTR area. For each person, there are 2 numbers of repeats in each VNTR region (one from mom and one from dad), so you are getting both counts. If you do this for a number of different VNTR regions, you can build a profile for a person that is statistically unique. The resulting DNA fingerprint can then be compared with the one left by the "perp" at a crime scene to see if there might be a match.

Here's how it works in general:

1. Isolate the DNA.
2. Cut the DNA up into shorter fragments containing known VNTR areas.
3. Sort the DNA fragments by size.
4. Compare the DNA fragments in different samples.

The way we sort by size is gel electrophoresis, and then we look at the results using a Southern Blot.



DNA Fingerprinting Step by Step

Now let's look at the exact steps used...

1. DNA is isolated from a sample such as blood, saliva, semen, tissue, or hair. DNA has to be cleaned up, because, unlike in a pristine laboratory, samples at a crime scene are often contaminated by dirt and other debris. Sometimes, DNA must be isolated from samples dried to patches of cloth or carpet, and getting the sample safely out of these fabrics adds additional steps to the isolation and purification processes.

2. The huge genome is cut up with restriction enzymes to produce short, manageable DNA fragments. These bacterial enzymes recognize specific four to six base sequences and reliably cleave DNA at a specific base pair within this span. Cleaving human DNA with one of these enzymes breaks the chromosomes down into millions of differently sized DNA fragments ranging from 100 to more than 10,000 base pairs long. You have to carefully select an enzyme that doesn't cut within any of the VNTR loci that are being studied; for RFLP analysis, the enzyme(s) chosen will ideally cut close to the end on the outside of a VNTR region.

3. These DNA fragments are then sorted by size using gel electrophoresis. In this process, DNA is loaded into a slab of Jell-O-like agarose and placed in an electric field. The DNA is separated by size because:
    - DNA, being negatively charged, is pulled through the gel toward the positively charged electrode.
    - Larger fragments move more slowly than smaller ones through the porous agarose.

    Once you have separated the DNA, you can determine the relative size of each fragment based on how far it has moved through the agarose.

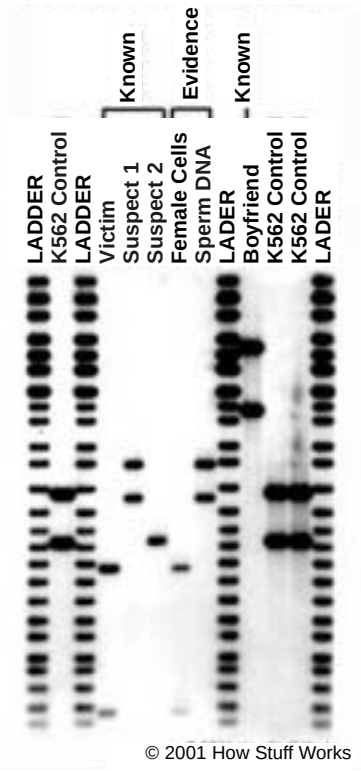
4. DNA fragments that have been separated on an agarose gel will begin to disintegrate after a day or two. To permanently save the DNA fragments in this segregated state, you need to transfer and permanently affix DNA to a nylon membrane. First, the DNA is denatured from its native double helix into a single-stranded state (this frees up nucleotides to base-pair with DNA probes for step 5 of the process). The positively charged nylon membrane is then placed on top of the agarose gel and used to sop up the negatively charged DNA fragment, like you might blot ink off a newspaper with Silly Putty.

5. Unlike Silly Putty, however, you can't actually see any of the DNA on your membrane. In order to figure out which fragments contain a particular VNTR locus, you have to flag them with some kind of tag that you can visualize. How do you do this? You simply make use of the basic structure and chemistry of DNA. DNA normally occurs as a double-stranded molecule, as two strings of nucleotides twisted around each other. The structure is held together by weak bonding between nucleotides on opposing strands. Only certain pairs of nucleotides can interact (adenine with thymine and guanine with cytosine), so these nucleotides are said to be complementary. To locate a specific VNTR sequence on a single stranded DNA fragment, you can find it by simply:
    - Making a DNA probe out of a DNA sequence complementary to that of a VNTR locus.

- Labeling the probe with a radioactive compound.
- Letting the probe bind to like DNA sequences on the membrane.
- Using the radioactive tag to find where the probe has attached.

6. Once the radioactive probe is stuck to its target on the membrane, you can take a picture of it using special X-ray film. You don't need a camera or other complex machinery to accomplish this feat --all you have to do is place the membrane against a special sheet of film for a short period of time! How does this work? In a regular camera, the film has a special coating that undergoes chemical changes when it absorbs the energy of a photon of light. When you take a picture with a camera, the light you let in by opening the shutter forms an image on the film. X-ray film, on the other hand, picks up radiation emitted from the natural decay of the isotope used in your probe. What you see on the film is a darkened band that indicates the places on the membrane where the probe has bound to DNA containing the VNTR sequence.

Sexual Assault Case



The results of RFLP analysis of one VNTR locus in a sexual assault case. DNA from suspects 1 and 2 are compared to DNA extracted from semen evidence. You can see in this sample that suspect 1 and the sperm DNA found at the scene match. Suspect 2 has a profile totally different from the semen sample; his DNA fragments have run much farther down the gel, meaning that they are shorter. You can also tell that he is a homozygote because there is only one, darker band indicating the presence of two copies of the same fragment. The other samples tested come from heterozygotes, because they have two bands of distinct sizes in each lane. DNA isolated from the victim as well as a human DNA (K562) that serves as a standard size reference are included as controls.

Playing the Numbers Game

The results from just one VNTR locus by itself don't pinpoint a suspect anymore than having one digit of someone's Social Security number (SSN) would let you figure out who they were. For example, a certain percentage of people are likely to have the number 2 as the first digit in their SSN. Similarly, for any given VNTR locus, a fragment length corresponding to a certain number of sequence repeats occurs in a certain number of individuals. What gives DNA fingerprinting its power is the combined analysis of a number of VNTR loci located on different chromosomes.

The final DNA profile is compiled from the results of four or five probes that are applied to a membrane sequentially. Each probe targets a different VNTR locus. Using four probes (as in the figure below) actually gives you eight pieces of information about an individual, since each of us has two separate copies of each VNTR region. To add to the complexity, it turns out that each VNTR locus usually has approximately 30 different length variants (alleles). Each of these alleles occurs at a certain frequency in a population. To get the probability that a given 8 band profile will occur, you multiply the eight different allele frequencies together.

Calculating a DNA Profile Frequency

Locus	Band Bin Frequencies Band 1/Band 2	Locus Frequency	Combined Frequency
1	0.08/0.02 (8%) (2%)	0.003 or 1 in 333	——
2	0.15/0.04	0.012 or 1 in 83	1 in 28 thousand
3	0.08/0.06	0.010 or 1 in 100	1 in 3 million
4	0.22/0.09	0.040 or 1 in 25	1 in 70 million

While the number of repeats at a single VNTR locus can't distinguish an individual from the rest of the population, the combined results from a number of loci produce a pattern unique to that person.

Using four loci, the probabilitythat you'd find a given allele combination in the general population is somewhere around 1 in 5,000,000. In the United States, the FBI incorporates 13 sites on average into its profiles. With 26 different bands studied, you'd be incredibly hard pressed to find two unrelated individual with the same DNA profile; the odds of a match in this case are well more than one in a hundred billion. The bottom line is that, unless you have a twin, you're statistically two thousand times more likely to win the Publisher's Clearinghouse sweepstakes (1 in 50,000,000) than to have a DNA profile that matches anyone else.

DNA Evidence in the New Millennium

In 1985, DNA entered the courtroom for the first time as evidence in a trial, but it wasn't until 1988 that DNA evidence actually sent someone to jail. This is a complex area of forensic science that relies heavily on statistical predictions; in early cases where jurors were hit with reams of evidence heavily laden with mathematical formulas, it was easy for defense attorneys to create doubt in jurors' minds. Since then, a number of advances have allowed criminal investigators to perfect the techniques involved and face down legal challenges to DNA fingerprinting. Improvements include:

- **Amount of DNA needed** – RFLP analysis requires large amounts of relatively high-quality DNA. Getting sufficient DNA for analysis has become much easier since it became possible to reliably amplify small samples using the polymerase chain reaction (PCR). With PCR, tiny amounts of a specific DNA sequence can be copied exponentially within hours.

- **Source of DNA** – Science has devised ingenious ways of extracting DNA from sources that used to be too difficult or too contaminated to use.

- **Expanded DNA Databases** – Several countries, including the U.S. and Britain, have built elaborate databases with hundreds of thousands of unique individual DNA profiles. This adds a lot of weight to arguments formerly based on mathematical theory alone, but it does raise questions of civil liberty as authorities ponder whether everyone in a community should be forced to submit a sample for the sake of completeness.

- **Training** - Crime labs have come up with formal protocols for handling and processing evidence, reducing the likelihood of contamination of samples. On the courtroom side, prosecutors have become more savvy at presenting genetic evidence, and many states have come up with specific rules governing its admissibility in court cases.

- **Science Education** - In recent years, a number of debates have erupted around the world over issues like using DNA evidence, cloning animals or selling genetically modified crops. It has dawned on many that to be active, informed participants in such ethical debates, the general public must understand the basic tenets of genetics, statistics, and the like. Students in some schools today aren't just learning about dominant and recessive genes in a lecture; they are performing PCR and RFLP analysis on samples to look for that recessive gene!

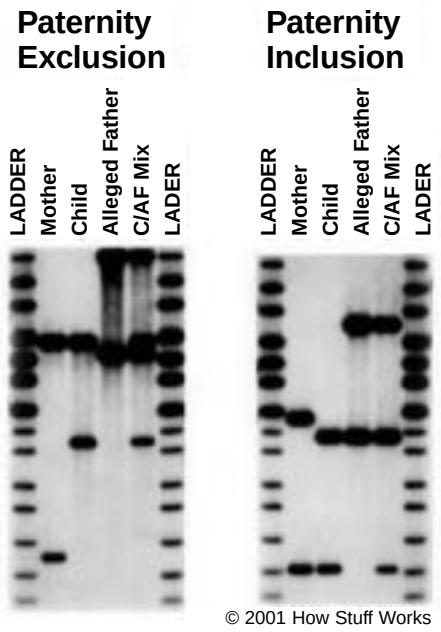
Using DNA Evidence

Given the high profile DNA evidence had during the O.J. Simpson trial, most people know DNA profiles are used by criminal investigators to:

- Prove guilt: matching DNA profiles can link a suspect to a crime or crime scene. The British police have an online database of more than 360,000 profiles that they compare to crime scene samples; more than 500 positive matches come up a week!

- Exonerate an innocent person - At least 10 innocent people have been freed from death row in the United States after DNA evidence from their cases was studied. So far, DNA evidence has been almost as useful in excluding suspects as in fingering and convicting them; about 30 percent of DNA profile comparisons done by the FBI result in excluding someone as a suspect.

DNA evidence is also useful beyond the criminal courtroom in:



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- Paternity testing and other cases where authorities need to prove whether or not individuals are related. One of the more infamous paternity cases of late revolved around a 1998 paper in the journal "Nature" that studied whether or not Thomas Jefferson, the third president of the United States, actually fathered children with one of his slaves (in case you're wondering, according the researchers, the answer is a resounding yes).

DNA evidence can pinpoint whether or not someone is a parent.

- Identification of John or Jane Does - Police investigators often face the unpleasant task of trying to identify a body or skeletal remains. DNA is a fairly resilient molecule, and samples can be easily extracted from hair or bone tissue; once a DNA profile has been created, it can be compared to samples from families of missing persons to see if a match can be made. The military even uses DNA profiles in place of the old-school dog tag. Each new recruit must provide blood and saliva samples, and the stored samples can subsequently be used as a positive ID for soldiers killed in

the line of duty. Even without a DNA match to conclusively identify a body, a profile is useful because it can provide important clues about the victim, such as his or her sex and race.

- Studying the evolution of human populations - Scientists are trying to use samples extracted from skeletons and from living people around the world to show how early human populations might have migrated across the globe and diversified into so many different races.
- Studying inherited disorders - Scientist also study the DNA fingerprints of families with members who have inherited diseases like Alzheimer's Disease to try and ferret out chromosomal differences between those without the disease and who are have it, in the hopes that these changes might be linked to getting the disease.