

Frederick Bieber
American Society of Law Medicine & Ethics
DNA Fingerprinting and Civil Liberties Project
RO1-HGH002836-02
Workshop 1 Presentation
May 14-15, 2004

Frederick Bieber

Fundamentals of human genetics
Past, present, future of DNA technology
Successes & pitfalls in forensic DNA profiling

Thank you for coming everybody. And thanks to Dorothy Wertz for putting this conference together. Really this was her brainchild and I must say that it's been a wonderful experience to work with Dorothy over a long period of time. Many of her colleagues are here that you might enjoy meeting and hearing about Dorothy. And, I think one of the most memorable things about her was her eclectic collection of jewelry. She used to come to - pretty much every lunch that I ever saw her at and have to ask, "Dorothy where on earth did you get that ring?" She would have a magnificent ring and she would say, "Well, Fred, this comes from Dubai. I was just there last week." She was an invited speaker all over the world and collected precious items, gems and gold and had a most unusual and characteristic way of wearing them and carrying herself; a real character, a brilliant scholar, and a wonderful colleague so it's an honor for me to participate in any activity that she had anything to do with.

If we could go to the first slide I want to remind all of you who aren't part of the DNA community and I must say that it's open. We have a few Harvard College students here today and some post doctoral fellows from other parts of the university to remind ourselves that the way we use the DNA molecule in the criminal justice system is only a very small component of how it may be used in hospitals and research labs around our country and around our university and certainly most of my work is in the upper left in the medical genetics arena. And I can tell you that many of the protocols that I'll briefly touch upon today are used as much or more in the medical context than they are in the forensic context. And there is a lot of cross-fertilization between medical scientists and basic research scientists in the forensic community and moving the technology back and forth across those borders.

As one example, I will just mention that patients that have suffered hemalogical malignancies like chronic myelomonocytic leukemia, acute lymphocytic leukemia and other leukemias often need total body radiation and bone marrow transplant in the patient and those bone marrow transplants usually come from unrelated matched donors. And over time they need to be followed sequentially, sometimes every three to six months, by bone marrow aspiration, either from the sternum or the iliac crest. So what proportions of the cells, the myelin cells producing lymphocytes and other cells in the blood, circulation have come from the donor as opposed to "the original cells?" The hope, of course, in this protocol is that the radiation will dry up the malignant cells and might be replaced by the healthy cells from the matched donor.

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We use in our own hospital the same equipment, the same instrumentation, the same radiation kits, and the same methods of interpretation identical in every way to what Brendan Shea from the FBI and Sharon Convery from the Mass. State Police, who are here today, use in matching DNA profiles or exuding DNA profiles at crime scenes. So many of these issues, of course, come up in admissibility hearings about whether some technology or technique is new or novel, and therefore does it past muster in the so-called Frey standard or the Daubert standard, and whether it is widely used in the community.

The amount of testing that goes on is well known to those of you in this field, but many who are students are not aware of the fact that there are literally tens of thousands of criminal investigations and many paternity cases - thousands, tens of thousands or hundreds of thousands of paternity cases which are generally civil and not criminal that make use of this technology; and the bottom line, I think, for the students in the room is the powerful exculpatory or exonerative power of DNA testing in the way it is used today by testing enough genetic locations or loci, which can exclude as many as a third of the individuals. In paternity cases, they have the wrong man as the potential father, or possibly the wrong suspect or the wrong arrestee.

Chris Aspen has made reference to the exonerations. As you probably know from hearing the news that since the first DNA based exoneration of someone who had previously been convicted in 1989 there have been well over three hundred exonerations in the United States alone for serious criminal offenses, including murder and sexual assault. And about half of those have been based on DNA testing which post conviction has shown that the two DNA samples, one from the known and one from the evidence, don't match. So there is a powerful exonerative potential for using this and it's just as important in the investigation to exclude a suspect as it is to include somebody.

We know that we inherit, in large part, most of the DNA that we are using in this technology--from each parent for the autosomal loci, half from Dad and half from Mom. And these genetic loci are located physically on these paired chromosomes. And this is a normal human male karyotype, showing the X and the Y in the lower right. Females have two Xs. Keep in mind that your friend, the Y chromosome--all the guys in the room have at least one of them. And this is transmitted, of course, only from father to son and grandsons. And there are a number of loci that are mapped specifically to the Y and they tend to move as a group, as a so-called haplogroup, on the Y chromosome because there is not a genetic exchange or a recombination in meiosis for much of the Y chromosome. There are several regions on the Y--the PR and QR at the tips here--that do exchange with their homologous DNA sequences on the X. But, by and large, there are regions on the Y that we can now use to track male lineage and we'll come back to that tomorrow in talking about other things.

The molecule itself, as you know, is a helix made up of four different nucleotides, A, T, C, and G and they pair in a specific way. And what the forensic biologists do is

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simply hit a sequence region of this Watson Crick double helix and literally learn the alphabet from left to right on both strands, sometimes in certain regions or to measure length differences between certain regions where there are repeat units of the DNA helix.

The next slide reminds us that most of the DNA in the human genome is non-coding. Only a small portion of it actually encodes functional molecules, or any molecules that are proteins that may have enzymatic or structural function. But this large amount of non-coding DNA includes the so-called STR, the short tandem repeats, or the VNTR, the variable number of tandem repeats, that are located in paired fashion as allele variants and we can simply track the number or size of the molecule itself and see how many of these tandemly repeated units there are as a way to produce a genotype for a particular individual sample.

However, most of the variation of the genome isn't related to these repeat units. They are actually variants of the single base pair, the single Watson-Crick base pair. So there are sequence differences. We refer to them nowadays as "snips," SNPs, or single nucleotide polymorphisms, which are known for the majority of the variation of the genome. The rest are deletions or tandem repeats or inversions where a segment of DNA has actually reoriented itself with regard to the centromere.

And this would be a typical three-person pedigree, mother the circle, the yellow square father, and the gray square son. And you can see just as a cartoon for a typical result of STR profiling in a single locus, both parents here were heterozygous, with different size repeat units. So mom has a seven repeat on one chromosome and a fourteen on the other. Dad has an 8/10. We refer to them as these numbers. Numbers refer to the STR lingo as the number of these tandem repeats. And clearly if we obey Mendel's laws for these STR loci, then a child, any child, would have one and only one allele or repeat size from each parent. In this case, he inherited a seven from the mother and a ten from the father. So if this were a paternity test and this man, the yellow man there, 8/10, whose name is the punitive father, you would say based on the results of the single locus example that he could not be excluded as a potential father. You couldn't say he was the father based on a single locus, but you could make a mathematical prediction of how commonly you would find somebody else with that profile.

In forensics, the simple role is to compare two samples, an evidence sample and a known sample. That known sample could be from a victim. It could be from a defendant or a suspect. Compare these profiles, these so-called STR profiles, from one sample to another.

And in doing so, the lab personnel simply take the known standard--it could be a blood sample stored like at a military database. All the soldiers have blood cards stored. If they are lost in the battle in Baghdad, then their body or body part from the Humvee

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that's exploded can be compared to a stain on that blood spot card. And the analyst simply crosses these samples in methods that we don't have time to discuss.

Basically, so here's a cartoon showing a little cutout of a blood spot, and anyone in the military has a card that looks like this stored in Rockville, Maryland. The protocol is fairly straightforward to allow DNA within the nucleus of these cells to be released and there you have a sample--in this case the victim could be a suspect.

PCR, for the students in the room, is the common method now used to amplify trace amounts of DNA.

And here are the ingredients of the PCR reaction that go in a small tube. Many of the students here have isolated DNA from plant or animal, and this is a very standard method that is used in all hospitals and research labs - simply to amplify blood with a lot of instrumentation that you are all familiar with - the heating block that heats and cools in typical fashion, duplicating what the cell does in nature to replicate the DNA molecule. In this instance, the specific primers are used, like the five and three prime regions of a gene, that will amplify very specific regions of the DNA that contain these tandemly repeated areas. So we aren't amplifying the whole genome but simply a targeted area shown here in color and after about thirty cycles of this PCR reaction which will just take a few hours time, one could have literally millions of copies of that trace source. So the single pair root around the cell that's found or pulled from the crime scene would contain enough DNA after PCR to produce a proper profile for comparison against a known sample.

All these samples have been processed with DNA methods that I just mentioned, including PCR, to look for these repeat regions. Even degraded samples, cigarette butts, chewing gum--any trace samples that you could imagine--would be potentially amenable to this method.

And again, what we are looking for are just the variable number of these tandem repeats or short tandem repeats that are typically four base pairs in length, but not necessarily. Any one of us in this room could be a 5/5 locus--both our mother and dad gave us five copies of this ATGG repeat. Or one of us could be a 3/4 or 3/5 or 4/5, so you can see that with a small number of alleles shown here at three, four and five, there are many pairwise combinations. So even with the testing of a small number of loci one can fairly efficiently exclude someone who isn't a contributor of the sample.

These loci are picked from around the human genome so that they can be considered independent of one another. And the statistical data on the frequency of these particular repeat sizes can be used and multiplied to make a statistical prediction of how common or how rare a complete profile might be in an individual of a certain racial or ethnic group. And that's typically what's done in court.

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The typical system looks something like this. There are some more modern instruments today, but this is a 310 that's widely used still today hooked up to a PC Mac usually.

The sampling trays allow roboticized processing of a large number of samples. And Ron Forme who is here from the RCMP is really a pioneer in developing high-speed automation for looking at literally thousands of samples in a weekend.

And, here is an example of what actually happens in the capillary, a small capillary shown here that will allow the PCR amplified products to pass through in accordance with their size. A laser beam hits them because we use fluorescently tagged PCR primers and the amplified PCR products will be able to be discerned from one another. And the end result of the process called an electropherogram. This is what the data actually looks like out of the computer that Brendan Shea and Kristn Koch would look at. They would call you and say, "We have evidence that the drinking glass found in the hand of the murder victim had saliva on it, and it had her saliva and also saliva matching one of the core suspects at that party, Dwight Carter, and there is a match at the FGA locus both twenty and twenty-one alleles were found." These are the repeat sizes again. At the VWA locus, the person is homozygous, has two copies of the fifteen alleles, and so on at the D3 locus. So typically nine, twelve and sixteen of these different loci – 1-2-3 - shown here are analyzed in a typical forensic case.

And these are just three shown – that's a typical result with a so-called match. The samples would be said to match. But the courts may not allow you to use that word 'match' depending on how swift your defense attorney is. He may or she may object to using that term.

I mentioned SNP's before and I just want to re-emphasize this. We will be talking about it tomorrow. SNPs are the newest horizon of forensic testing because there are probably five or ten million of these variations in the human genome at the level of the single Watson-Crick base pair. As I said earlier, the most common form of genetic variation at the base pair and automotive profiling will allow one to get data not of ten, thirteen or sixteen loci but literally on tens of thousands. Again, these SNPs are inherited. When they are inherited on the autosomes, they are inherited from each parent and there is a slightly different nomenclature for using the SNPs. As I pointed out earlier that friendly Y-chromosomes on the lower right is inherited only from father to son.

So brothers who are related from common ancestors all have the same Y chromosome and this obvious difference between Y inheritance and autosomal inheritance can be useful in narrowing down a scope of an automated computerized search. Let's just do Y SNP profiling. As I mentioned, those Y SNPs are inherited as a block together. And one could look at Y haplotypes and--an intriguing thought I had is

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that maybe you'll find a very rare Y SNP haplotype associated with a very rare surname, Bieber or Asplen. And I wonder if someday whether the home office in Britain will be knocking on doors of everyone named Asplen because the crime scene sample was processed with Y SNP haplotype methods and we found that this very, very Y SNP profile was only seen typically in men named Asplen. So let's send someone through the phone directory looking up all the Asplens and let's just knock on their doors and bring them in as suspects. An intriguing idea that I would like you to think about because we inherit surnames from our fathers as well as a Y chromosome.

I shouldn't forget the mitochondria. In wrapping up, the mitochondria is inherited let's be fair to the moms. The moms gave us not only a lot of patience when we grew up but they also gave us their mitochondrial DNA inherited only from a mother to all her children. So boys would have inherited the mitochondrial chromosome from mom but not pass it on. Only the sisters would pass it on. There is particular region of general interest shown in blue here where sequences in variable regions occurs. And one could compare the mitochondrial sample in let's say in the shaft of a hair cell where there is no nucleus and process crime scene samples even that are anucleic simply because there still may be mitochondria DNA available.

Again this is would be a pedigree showing the maternal lineage transmission of mitochondrial chromosomes from the mothers to all of her children but only passed on from those females to their sons or daughters.

I want to finish up in the next minute and half by mentioning some new technology. This is exciting but sort of old school. The way we want to separate epithelial cells, in a mixture from a rape kit, from the sperm cells is by using a differential extraction method. This is a gentle extraction to release the DNA from the epithelial cells followed by a more rigorous extraction. And this so-called differential extraction would separate the sperm DNA from the vaginal DNA from a rape kit swab. And this is the way it is done most of the time.

But there are some new methods that we are developing at our own hospital and in collaboration with the New York City Medical Examiner to use something called laser capture microscopy, or LCM, which is widely used in pathology research but only recently applied to forensic samples. This is a real case that came to Brigham and Women's Hospital from the DA's office in Suffolk County where a young girl was sexually assaulted, and was pregnant, had a miscarriage and the aborted (unclear), the POC, product of conception was sent to Cellmark and they called the DA, Kelley Downs, and said all we are getting is his mother's profile. Well, Kelly phoned me up and I said, "They probably just have all the residual cells from the abortion procedure. Send the paraffin block back to us with an order and we'll use LCM on it."

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This reminds you of what we all look like as embryos. Here is a beautiful embryo, human with the eye here and the arms and legs. Inside, the amniotic sac--and all these cells out here are chorionic villi which will invade the uterus in the form of placenta. When DNA procedures are performed after women become pregnant after rape, one will often get these villi which are fetal in origin, would have a fetal karyotype, and therefore be an important piece of evidence. But they may also get the maternal tissue from the uterus.

And in the histological image we'd see villi here on the right. These are all villi that are projections into the uterus or the placenta. And these are the blood vessels and muscle tissue and epithelial cells of the maternal placenta.

So these are all villi, and we'd like the state police to collect and send them to Cellmark, but sometimes they will send you a mixture. And PCR leads to quantity that will amplify immediately the most abundant cellular material so you'll often get mom's profile.

There are a number of instruments now, at least four. One called Arcturus. One called Molecular Machines, Inc., and another called P.A.L.M. Another one that Lykes is coming out with allows you to use pulse laser beams in a set up something like this, and project the pulse laser around the tissue of interest, lift it up on the snap cap tube and put just that cell or cell type into a tube and do the DNA extraction like that. This targets very specific cells or tissues were DNA extraction, alleviating this problem of mixtures and erroneous results.

This case went to trial in late '02, based on our post doc, Mary (?)'s wonderful work with Jeff Hickey at Cellmark.

And here is showing another image of pulse laser cutting out. What do you see here? A dark nucleus and a tail, those are single sperm cells which we can literally carve out from a mixture on a pap smear for example and catapult in this system of P.A.L.M., Pulsing System Laser Microscopy. So this is a new method, again having its roots and use in pathology labs, for separating tissue types for basic research but has obvious application to trace samples in the forensic community.

There are two more sperm cell nuclei. (At this point, the video and the online multimedia video contain sound malfunctions)

...in the 90's from a group called Technical Working Group on DNA Analysis Methods, or TWGDAM. That acronym that's morphed into SWGDAM, which succeeds most recommendations that the FBI DNA Advisory Board made in the 1995 to 2000 era and all the labs that receive Federal funding have to operate according to rules and regulations put forth by SWGDAM along with the American Society of Crime Lab

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Directors, ASCLD, where inspections of these labs submit to every year. CAP, the College of American Pathologists produces proficiency testing modules for these labs and they are fairly rigorously scrutinized in that way. ABC stands for the American Board of Criminalists, where a fair number of individuals work in crime labs actually take the time and trouble to jump through that hurdle and become certified individually by that particular organization.

Really closing now. Let's be sure that we are all on the same wavelength about what DNA does and doesn't tell us because there are some obvious caveats that I think we are all aware of. DNA mixtures being particularly a troublesome issue in certain instances, the statistical interpretation of mixtures can take a whole day to discuss. But there are many instances, and I should mention twins as one of them. About one in 260 individuals are monozygotic or identical twins; therefore a DNA profile is no good in differentiating one twin from another and from close relatives. Dave Lazer will tell you in this conference – it may be somewhat problematic because the brothers may have very similar profiles as well. So if there's a degraded DNA sample and you only tested six or seven loci and you have an inclusion, my first question would be is that person a twin.? Does he have a brother or close relative that you might need to consider excluding? Because you certainly don't want to implicate the wrong person based on a partial profile match. Similarly, not all exclusions are true exclusions. We won't take the time to worry over those interesting cases, but one has to consider DNA evidence in a specific context in the case and not slavishly believe that a match is necessarily probative or an exclusion is necessarily probative.