GUEST EDITORIAL

George F. Sensabaugh,[1] *D.Crim.*

Forensic Biology—Is Recombinant DNA Technology in its Future?

Forensic biology—paternity testing and the testing of biological evidence materials—has advanced dramatically over the last 15 years. Much of this progress has been fueled by the discovery of many new genetic polymorphisms among human proteins. A number of these new polymorphisms have worked their way into the forensic science repertoire with the result that more definitive genetic typing information can now be provided to the legal system. There has been a concomitant broadening of the technological base of the field as electrophoretic techniques have taken their place beside the traditional immunological and chemical testing procedures. These developments are all well documented in Gaensslen's *Sourcebook* [*1*].

It is now pertinent to ask whether forensic biology should prepare itself to take advantage of a new category of genetic polymorphism—polymorphisms at the deoxyribonucleic acid (DNA) level. The first DNA polymorphisms were noted in the late 1970s and, since 1980, some 200 such polymorphisms have been defined [*2*]. The potential applications in forensic biology are obvious and several laboratories have begun working in this area. The first glimmerings of promise are offered in several recent papers, two of which appear in this issue of the *Journal* [*3-6*]. The objective of this commentary is to describe briefly the technology associated with the detection of genetic variation at the DNA level and then to consider what barriers have to be overcome if this technology is to pass into the mainstream of forensic science.

Genetic Variation at the DNA Level

The first step in the detection of DNA polymorphisms is the isolation of DNA from the source material. This DNA is subjected to controlled fragmentation using bacterial enzymes, called restriction enzymes, which cut double-stranded DNA at sequence specific positions along the double helix. This reduces long DNA molecules to a reproducible set of shorter fragments usually ranging in length from several hundred to several thousand base pairs. These restriction fragments (RFs) can be separated on the basis of size by electrophoresis on agarose or acrylamide gels.

A very large number of RFs are produced by digestion of the human genome with even a single restriction enzyme, and detection of any specific fragment requires the use of a hybridization probe, a labeled piece of DNA that binds specifically to the target RF by complementary base pairing. Hybridization probes are conventionally labeled with radioactive isotopes

[1]Associate professor of forensic science, Forensic Science Group, Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA 94720.

(usually ^{32}P), but increasing attention is being given to the development of nonisotopic probes. The procedures for DNA digestion, electrophoresis, and hybridization are described in several texts (see, for example, Ref *7*). They are not complex and have been used successfully in undergraduate biochemistry and genetics teaching laboratories.

Differences among individuals are expressed as restriction fragment length polymorphisms (RFLPs). RF length differences may result from several kinds of genome level differences. Mutations altering the base sequence at a restriction enzyme recognition site can result in a loss of the site. Similarly, mutation can generate a new cleavage site. Insertion or deletion of blocks of DNA between the boundaries defined by two cleavage sites also changes RF lengths. Some regions of DNA contain multiple segments of short sequence repeats and there is a class of RFLPs that differ in the number of repeat segments present.

RFLPs have been found both in nuclear DNA and in mitochondrial DNA. Nuclear DNA RFLPs are inherited as simple Mendelian characters. Most show simple one-band homozygote and two-band heterzygote patterns. RFLPs resulting from the gain or loss of a restriction site generally have few alleles, whereas the tandem repeat variants often have many [*2*]. Multiple band patterns occur when the probe hybridizes with more than one RF; this often reflects gene duplication. Mitochondrial DNA RFLPs have different genetics because they are maternally inherited [*8*]; their potential forensic science value is thus limited to evidence analysis.

A special case of multiple band RF patterns has been described by Jeffreys et al [*5,6*]. They have used probes against repeat sequences duplicated at multiple loci in the nuclear genome. Their RF patterns contain as many as 50 bands of variable intensities. The elements of these patterns appear to behave as Mendelian characters. Jeffreys et al suggest that these complex RFLP patterns are individual specific DNA "fingerprints." They note that these may have forensic science utility and have recently published a paternity test case [*6*].

Conditions for Forensic Science Utilization

Before any genetic marker can be used in the forensic science context, whether in paternity testing or in evidence analysis, certain conditions must be met. These conditions are part of the foundation for legal acceptance and include the following:

1. The marker used must be validated as a true genetic character; its mode of inheritance must be demonstrated by family studies and it must be shown to be stable in an individual over a lifetime. The use of RFLPs in genetic linkage studies provides this validation.

2. Gene frequencies for the major population groups must be established to provide a base for the interpretation of findings. For simple RFLPs—those with no more than a few alleles—tests on a few hundred individuals should be adequate to establish allele frequencies. As the number of alleles increases, so also does the number of individuals to be tested to establish reliable allele frequencies. Population data on most of the known RFLPs is deficient at this time.

3. There must be a nomenclature to define the variants in each polymorphic system. This is necessary both for purposes of record keeping and for communication of data. The importance of a nomenclature system is additionally evident in evidence analysis situations when a sample from an unknown individual must be genetically profiled. This condition is not a problem for RFLPs; variants are identified by their molecular weight.

4. Standard types and typing reagents must be available to the forensic science community to allow independent testing in different laboratories. The central problem here is the availability of hybridization probes. At present, no RFLP probes are commercially available although several such probes are due on the market shortly. Access to some probes may be blocked by proprietary interests; this will limit forensic science utilization.

5. Methodological guidelines need to be defined to insure reliability of test results. These guidelines would include standard good practice in genetic testing: the use of appropriate

standards and controls and so forth. RFLP testing may need special controls, for example, a control to determine the completeness of the restriction digestion; the need for such controls will show up as experience is gained with the typing procedures.

6. Ultimately, markers must pass the barrier of blind trial testing. This establishes that the marker does not possess inherent ambiguities that might lead to typing error. The nature of the blind trials depends of course on whether the targeted use is paternity testing or evidence analysis.

The foregoing conditions are a distillation of our experience with the existing body of blood group and protein polymorphisms. The simple RFLPs give no indication of behaving any differently and satisfaction of these conditions, although time-consuming, should be fairly straightforward.

The situation with Jeffreys et al's DNA "fingerprints," however, is a different matter. The uniqueness of these "fingerprints" can be truly established only by testing of all individuals, both living and dead. Clearly this is not possible, yet anything less leaves open the hypothetical possibility of an unobserved duplication. The alternative is to develop a better understanding of the genetic processes underlying the generation of the complex patterns; this would allow an estimate to be made of the chance of a random duplication. This would in itself require extensive family and population studies. Additional problems are posed by the difficulty in establishing a nomenclature to describe the complex patterns of the "fingerprints." A possible approach here would be to convert the patterns to densitometric "spectra"; these could be classified in much the same way as infrared (IR) or mass spectra. Finally, the matter of methodological guidelines would need careful investigation; one intuitively expects complex patterns to be difficult to reproduce.

Application to Biological Evidence Samples

Two additional concerns must be addressed with regard to the application of RFLP testing on biological evidence samples: the adequacy of the amount of DNA in typical evidence samples and the stability of DNA in such samples.

The DNA content of evidence samples is at present a limiting constraint. Current technology requires 1 to 10 μg of DNA for a single analysis. Blood contains 5000 to 10 000 nucleated cells per microlitre; this corresponds to 25 to 50 μg of DNA/mL. Thus bloodstains would have to contain at least 50 μL of blood to be amenable to analysis. The corresponding limit value for semen is about 10 μL. To put this latter value in context, a vaginal swab holds about 100 μL of fluid; thus semen collected on swabs cannot be diluted more than about 1:10. These threshold values are not very encouraging since tests for many of the currently used genetic markers are more sensitive. However, the DNA technology is advancing rapidly and improvements in sensitivity can be expected.

Assessment of the stability of DNA in evidence materials centers on two questions: does DNA survive in high molecular form and are restriction sites modified in any way? The two papers in this issue of the *Journal* provide evidence that high molecular weight DNA can survive under conditions typically experienced by evidence samples. Moreover, the recovery of DNA fragments from mummy tissue and from 100-year-old dried skin is testament to chemical stability in the dry state [*9,10*]. Rigorous definition of the limits of survival requires measurement of hydrolysis rates as a function of moisture content, chemical environment, temperature, and exposure to light and other radiation. Endogenous nucleic acid digesting enzymes and nucleases and restriction enzymes produced by contaminating bacteria are unlikely to be a problem since they would be isolated from the DNA in cell nuclei by several membrane barriers; this, however, needs to be verified.

The question of restriction site modification can be answered in part from the known chemistry of DNA. It is known, for example, that ultraviolet light induces pyrimidine dimer formation and that both cytosine and adenine spontaneously deaminate to form modified

bases. The issue then is not whether modifications occur, but rather whether their occurrence affects RFLP analysis. This must be determined by experiment.

If DNA is demonstrated to maintain acceptable integrity in evidence materials, then the whole range of probes are open for use. There is nothing in the chemistry of DNA to suggest that different RFs should differ in stability. This is in contrast to the protein and antigen genetic markers, each of which has its own characteristic stability properties.

Conclusion

The purpose of this commentary has been to look at a possible future direction of forensic biology—the application of DNA technology in genetic typing analysis. The potential benefits are considerable; this technology may allow virtual identification of an individual. However, at this point in time, there are still significant barriers to overcome. Probes need to be made readily available; these probes need to be nonradioactively labeled since few forensic science laboratories have the facilities for isotope work. Current RFLP detection sensitivities need to be improved to justify application to evidence materials. Population data for RFLPs need to be generated. The stability of DNA in evidence materials needs to be critically assessed. It seems inevitable that these barriers will fall as the technology advances and as more experience is gained. This will leave only the major barrier to the acceptance of any new technology: inertia. We should begin to prepare now.

A recent paper by Gill et al. provides additional evidence that DNA maintains acceptable integrity in evidence materials [*11*]; they demonstrated that DNA "fingerprints" could be developed from bloodstain and semen stain samples. See also the commentary by Dodd [*12*].

References

[*1*] Gaensslen, R. E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, United States Department of Justice, National Institute of Justice, Washington, DC, 1983.

[*2*] *Proceedings of the Eighth International Gene Mapping Workshop. Cytogenetics and Cell Genetics*, Vol. 40, 1985, in press.

[*3*] Kanter, E., Baird, M., Shaler, R., and Balazs, I., "Analysis of Restriction Fragment Length Polymorphisms in DNA Recovered from Dried Bloodstains," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986, pp. 403-408.

[*4*] Giusti, A., Baird, M., Glassberg, J., Pasquale, S., and Balazs, I., "Application of DNA Polymorphisms to the Analysis of DNA Recovered from Sperm," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986, pp. 409-417.

[*5*] Jeffreys, A., Wilson, V., and Thein, S., "Individual Specific 'Fingerprints' of Human DNA," *Nature*, Vol. 316, 1985, pp. 76-78.

[*6*] Jeffreys, A., Brookfield, J., and Semeonoff, R., "Positive Identification of an Immigration Test-Case Using Human DNA Fingerprints," *Nature*, Vol. 317, 1985, pp. 818-819.

[*7*] Maniatis, T., Fritsch, E. F., and Sambrook, J., *Molecular Cloning. A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982.

[*8*] Cann, R., Brown, W. M., and Wilson, A. C., "Polymorphic Sites and the Mechanism of Evolution in Human Mitochondrial DNA," *Genetics*, Vol. 106, 1984, pp. 479-499.

[*9*] Paabo, S., "Molecular Cloning of Ancient Egyptian Mummy DNA," *Nature*, Vol. 314, 1985, pp. 644-645.

[*10*] Higuchi, R., Bowman, B., Freiberger, M., Ryder, O., and Wilson, A. C., "DNA Sequences from Quagga, an Extinct Member of the Horse Family," *Nature*, Vol. 312, 1984, pp. 282-284.

[*11*] Gill, P., Jeffreys, A. J., and Werrett, D. J., "Forensic Application of DNA 'Fingerprints'," *Nature*, Vol. 318, 1985, pp. 577-579.

[*12*] Dodd, B. E., "DNA Fingerprinting in Matters of Family and Crime," *Nature*, Vol. 318, 1985, pp. 506-507.