Letters

DNA Fingerprinting

When science and the laws of evidence come together, confusion can sometimes result, and some of that confusion is apparent in Colin Norman's article "Maine case deals blow to DNA fingerprinting" (News & Comment, 22 Dec., p. 1556).

Confusion in the case was generated when the prosecution asked that a key autoradiograph be resized, even though it was simply a cleaner version of an earlier one. Both autoradiographs were produced from the same nylon membrane on which DNA fragments had been permanently bound. Lifecodes took the position that, from a scientific laboratory point of view, resizing was clearly not necessary because the two autorads were simply two pictures of the same thing.

More confusion was generated when the defense attacked the reading of autorads that displayed a bandshift. The contention was that such autorads actually proved a nonmatch, because the probes did not line up exactly opposite each other. Muddying the waters regarding the interpretation of scientific test results is a normal defense tactic, and the reading of DNA autorads is no exception. Most judges and juries are not scientifically sophisticated, and defense attorneys attempt to capitalize on this fact.

Norman's article correctly points out that the need to demonstrate bandshifts (mobility differences) has been known for some time. This was done in the McLeod case by using monomorphic probes—a technique recommended by the scientific community. Michael Baird, director of forensic testing at Lifecodes, did not testify that any monomorphic probe would have shown a bandshift of 3.15%. Baird's testimony was simply that any monomorphic probe could be used to demonstrate the *existence* of a bandshift.

In fact, for the DXZ1 probe used, the mobility shift between evidence and exemplar was shown to be 3.37% at known 2-kilobase locations and 3.15% at 4-kilobase locations. Baird chose to use the 3.15% value for his calculations. This was the more conservative choice, because it was the smaller correction factor.

In terms of DNA litigation, the Maine case does not have legal precedent value. No decisions were handed down. However, the case is extremely important because of its educational value. It demonstrates how critically important it is for the legal and scientific communities to communicate effectively.

We at Lifecodes are working to improve our communications with our attorney clients. The communications problems with the Maine prosecutor have been ironed out, and Lifecodes is currently working on two other Maine cases. Lifecodes is also working with a number of attorneys, including several prosecutors, to put together a DNA litigation package. We welcome any input and suggestions from others in the legal-scientific community regarding the contents or structure of this kind of information package.

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While Southern blotting is technically robust, like any other scientific technique it has some known limitations when it is used in a particular way. The limitations arise from the fact that the migration of DNA bands across an agarose gel depends significantly on (i) the size of the bands, (ii) the composition of the gel (the concentration of agarose), and (iii) the running condition (faster versus slower). In certain conditions and given the expected location of the desired bands on a gel, two DNA bands differing in size by five to ten nucleotides, a few hundred nucleotides, or even a few kilobases (in pulsed-field gel electrophoresis) may appear identical in size. In cases where a difference of only one nucleotide between two samples may be sufficient to label an individual innocent or guilty, such inherent limitations are not acceptable. Moreover, two apparently identical DNA bands might differ in nucleotide sequence composition. The best conclusion that can be drawn from the apparently identical bands in a Southern blot is that they are "very similar" (meaning they have a high but "unknown" sequence similarity); determining whether they can be scored as "identical" (which is what the court wants to know) requires more work.

This does not mean that the DNA fingerprinting technology has received a fatal blow. Methods based on the polymerase chain reaction (PCR) amplification of numerous hypervariable sequences already exist that have potential for addressing all of these limitations. Some approaches demand particular attention because investigators have used a sequencing gel to analyze material obtained after PCR amplification of a hypervariable sequence (*1*). The advantage of using a sequencing gel rather than an agarose gel is that bands differing in length by only one nucleotide can be unambiguously detected. One can thereby eliminate cross-interpretations related to the "bandshifting" phenomenon. In addition, identical (in length) bands between two samples obtained through such a maneuver should be sequenced so that one can see whether these are 100% identical in nucleotide sequence composition. The bands of interest could be directly sequenced by any of the methods now in use (*2*). A deviation of this parameter from 100% would mean that the bands are nonidentical, even though they display *length identity* in a sequencing gel.

Another potentially important problem is the possibility of inadvertent (or even intentional) cross-contamination of samples. A possible check would be to perform every experiment by intentionally mixing the two samples in question in addition to handling the samples independently.

The following would be a prudent and inexpensive way to eliminate the need for obtaining data from multiple laboratories. Two DNA fragments (having no sequence similarity in humans) could be constructed that differ in length but are PCR-amplifiable by one set of primers only. Before any experiment is performed, one of these DNA fragments could be routinely included with the "sample" DNA and the other included with the "suspect" DNA. All the experiments then could be carried out with these "internally tagged" samples. This type of internal control has the potential for detecting inadvertent as well as intentional cross-contamination of samples.

It is difficult to understand why Lifecodes chose to go ahead with the Southern blotting technology to generate their data for the McLeod case without exploration of other more powerful techniques. Whatever the reason, I believe the recent episodes concerning DNA fingerprinting will have a positive effect on the future of this field.

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REFERENCES

1. J. L. Weber and P. E. May, *Am. J. Hum Genet.* **44**, 388 (1989); M. Litt and J. A. Luty, *ibid.*, p. 397; D. Tautz, *Nucleic Acids Res.* **17**, 6463 (1989); G. Sarkar and S. S. Sommer, in preparation.
2. C. Wong *et al.*, *Nature* **330**, 384 (1987); E. S. Stoflet, D. D. Koeberl, G. Sarkar, S. S. Sommer, *Science* **239**, 491 (1988); U. B. Gyllensten and H. A. Erlich, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7652 (1988); R. G. Higuchi and H. Ochman, *Nucleic Acids Res.* **17**, 5865 (1989).

Although the causes of bandshifting in DNA fingerprinting are generally appreciated, the procedures to correct for this effect (use of monomorphic probes to calculate bandshift correction factors) are inadequate. Rather than attempting to *correct* for band-

shifting, it would seem eminently preferable to *eliminate* it.

With respect to a potential solution to the bandshift problem, I note that this kind of problem is not unique to DNA fingerprinting. Whether samples are correctly identified is often tested chromatographically in many fields, for example, identical samples are required to have identical retention times on high-performance liquid chromatography or gas chromatography, or identical retention values on thin layer chromatography. To obviate any "bandshift" problems in these analyses it is common practice to coinject or co-spot a mixture of the two samples as well as to compare separate chromatograms or lanes. Similarly, co-electrophoresis of a mixture of the two DNA samples in a single lane of the gel [flanked on either side by lane(s) containing the individual samples] would eliminate the bandshift problem. In the mixed lane, each sample would experience the *same* set of factors affecting migration, whether it be sample load, impurities, gel or field inhomogeneities, and so forth. Thus, no correction factors are required and the criterion for a match would reduce to the finding, under visualization with each probe, of band correspondence in the two individual lanes and only single bands with no discernible band broadening for each band in the mixed lane. Conversely, in the event significant broadening or a doublet is observed at any of the bands, one could be quite certain that the two samples are nonidentical.

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The problem of bandshifting in Southern blots of genomic DNA digests is not unfamiliar to researchers. However, the solution that Lifecodes opted for is somewhat puzzling. Since DNA does not migrate as a linear function of fragment length in agarose gel electrophoresis (migration is inversely proportional to the log of the molecular weight), it seems overly simplistic to apply a single percentage correction to all the bands in a given lane of the gel. A simple yet better solution would be to spike each DNA sample with a set of marker fragments, for example, Hind III–digested λ DNA, and use these internal markers to calculate fragment sizes in each lane of the gel separately by linear regression. The bands from 0.5 to 1.0 micrograms of marker DNA would be visible above the background smear of genomic restriction fragments by ethidium staining. Alternatively, nanogram quantities of the markers could easily be detected by means of autoradiography by stripping and reprobing the blots with a λ-specific probe. Cross-hybridization between human probes and the marker would not be a problem as long as the human probe sequence was separated from its prokaryotic vector DNA. This method obviates the need to use several monomorphic probes in order to correct for the nonlinear bandshifting over the length of the gel.

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Erratum: In the report "The response of living cells to very weak electric fields: The thermal noise limit" by J. C. Weaver and R. D. Astumian (26 Jan., p. 459), figures 2 and 3 on page 461 were inadvertently interchanged. The captions were correct.