lele assignments, owing to (1) an apparent transcription error in the report and (2) an unknown difficulty with one of the parental DNA samples.

This project was the result of a year of enthusiastic informal discussions among the directors of the DNA diagnostic laboratories in the region. All participating members agreed that the project both identified potential shortcomings in current laboratory practices and provided a basis for comparing the technical and interpretive methods used by the various laboratories.

Recommendations for future testing included possible use of blood or cell lines from actual affected families, especially for those laboratories performing cystic fibrosis analysis. In addition, participants indicated that a rigorous definition of the components of the report be established to evaluate both interpretation of results and communication to referring health professionals. Directors expressed an interest in participating in the newly established national proficiency-testing program sponsored by the Council of Regional Networks (CORN). This program would provide a source of samples with previously established RFLP markers and an independent evaluation of allele and genotype assignments. However, the members also expressed interest in requiring additional information from the laboratories which would be evaluated at the regional level. The educational value of regional discussion of results and problems was felt to be too valuable to forgo, despite the availability of a national program. The value of an independent regional program has been reinforced by the decision of CORN to discontinue support of the national DNA proficiency-test program.

This DNA diagnostic-laboratory evaluation project joins the successful cytogenetic and biochemical genetic proficiency-testing programs currently offered by SERGG. The southeastern region is the only area of the country which provides such an extensive program for the genetics centers of the region. The authors are proud of the record of achievement in quality control of genetic services which has been established in the southeastern region. We hope this report will provide impetus to other regions to organize similar inter- or intraregional quality-assurance programs.

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Diagnostic Heteroduplexes: Simple Detection of Carriers of a 4-bp Insertion Mutation in Tay-Sachs Disease

To the Editor:

In the recent letter, "A PCR Artifact: Generation of Heteroduplexes," Nagamine et al. (1989) described the formation of heteroduplexes when homologous loci differing by an 18-bp deletion were amplified by PCR and analyzed by PAGE. They suggested that heteroduplex formation would allow the detection of heterozygotes for other loci or alleles if they contained a large enough deletion or insertion. We have observed heteroduplex formation in heterozygotes for an allele containing an insertion mutation of only 4 bp.

Approximately 70% of Ashkenazi Tay-Sachs disease is caused by a 4-bp insertion mutation in the *HEX A* gene encoding the α-subunit of hexosaminidase A (Myerowitz and Costigan 1988). To detect this mutation, we used oligonucleotides to amplify a 169-bp segment of DNA flanking this site. The PCR product was digested with the restriction enzyme *Hae*III to create a fragment small enough to allow differentiation between normal and mutant sequences after PAGE (fig. 1). In only heterozygous individuals, two bands, which migrated more slowly than the primary product, were observed in the lane containing undigested material (lane 3). An extra, faint band, just above the 47-bp insertion-containing fragment, was also seen in the lane containing digested product (lane 4). The two bands, "the doublet," migrating more slowly than the amplified product in lane 3, were found to be characteristic and

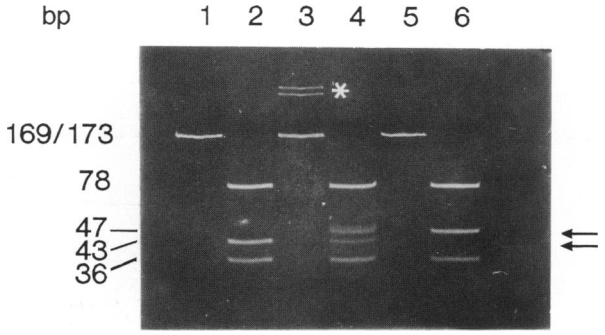

Figure 1 PCR products from individuals who do not carry the 4-bp insertion mutation (homozygous normal, lanes 1 and 2), who carry one copy of the 4-bp insertion mutation (heterozygous, lanes 3 and 4), or who carry two copies of the 4-bp insertion mutation (homozygous mutant, lanes 5 and 6) were electrophoretically separated on an 8% polyacrylamide gel. Lanes 1, 3, and 5 contain the undigested samples, while lanes, 2, 4, and 6 contain the samples digested with the enzyme *Hae*III. Arrows indicate the 43-bp fragment and the 47-bp fragment that results when the 4-bp insertion mutation is present in the 43-bp fragment. An asterisk denotes the heteroduplex bands formed only in the heterozygous individual.

predictive of the carrier state for the 4-bp insertion allele. The extra band in the digested product cannot normally be differentiated from the 47-bp band. These bands appear to correspond to heteroduplexes formed by the annealing of normal and mutant amplification products.

Nagamine et al. observed that the heteroduplex products of their amplified alleles were visible on polyacrylamide gels but not on agarose gels. Our results for the 4-bp insertion bear this out. Nagamine et al. suggested that such heteroduplexes produce a secondary structure that slows migration through polyacrylamide gels. Further, they suggested that the rate of migration is dependent on the base composition of the heteroduplex, hence the detection of two types of molecules depending on which pair of heterologous DNA strands anneal.

Nagamine et al. hypothesized that the heteroduplexes could be annealed along their entire length or only on one side of the deletion, leaving their ends free. The ability to cleave our product to completion with a double strand–specific restriction endonuclease (fig. 1, lane 4) indicates that the heteroduplexes are annealed throughout their length.

Our results show that deletions or insertions as small as 4 bp can allow formation of heteroduplexes that can be detected on polyacrylamide gels. We note that the effect on migration of the bands is more pronounced in the 169-bp product than in the 47-bp digested product. However, by increasing the concentration of polyacrylamide, the retardation of small heteroduplexes could be accentuated. For example, the 47/43-bp heteroduplexes could be resolved into two distinct, more slowly migrating bands in relation to the 47-bp duplex by using 12% polyacrylamide (data not shown). Heteroduplex-based detection of small insertions and deletions may be useful in the development of DNA diagnostics as well as in the identification of new mutations.

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