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Analysis of 'color PCR' by automatic DNA sequencer

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Recently Chehab and Kan developed a polymerase chain reaction (PCR)-based color complementation assay for the detection of DNA mutations (1). In this assay, competition between amplification primers conjugated to different fluorescent dyes is reflected by the color of the PCR products. We have developed a means for analyzing color-PCR products with an automated DNA sequencer (ABI Company, Foster City, CA) and used this procedure to detect the point mutation 1226 of the glucocerebrosidase gene (2,3).

Two 19 nucleotide primers identical except for a single centrally located base corresponding to the mutation site were synthesized. Rox (Rhodamine) and Fam (Fluorescine) (ABI) were conjugated to the 5′ ends of the primers specific to the normal and mutant sequences respectively. PCR was performed using a mixture of these primers and an unconjugated 20 nucleotide antisense-specific primer (4). To compensate for unequal emission intensities from the two dyes, the FAM-conjugated primer was diluted 1:15 with an unconjugated primer of the same sequence. One μl of a 1:2000 dilution was mixed with 5μl formamide and loaded on a 6% sequencing gel. Data obtained from individuals normal, heterozygous and homozygous for the mutation are shown in Fig 1.

The proposed modification: 1) Obviates the need to separate free primers from the PCR products; 2) permits quantitation of the amplified DNA by calculating the ratio between the competing peak heights; and 3) facilitates simultaneous analysis of multiple color amplification products from a single PCR in one gel lane.

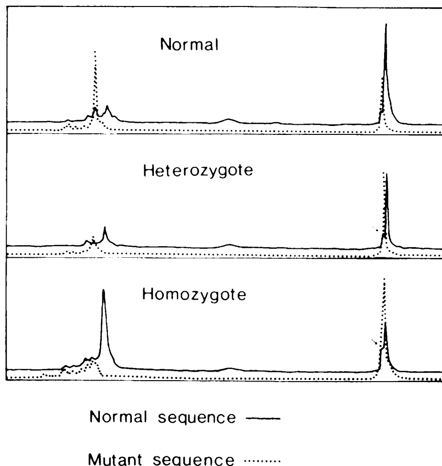

Figure 1: Results representative of the 3 patterns obtained from 17 different PCRs. The mean ratios (range) between the peak heights of the PCR products (on the right) were normal 2.6 (2.3-2.8), heterozygous 1.03 (1.0-1.04) and homozygous 0.2 (0.1-0.26). Migration differences due to the different dyes enabled us to detect slight spectral overlap in the Rox channel (↘) and to distinguish it from cross hybridization. Reverse overlap was not detected.

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