PCR test for cystic fibrosis deletion

SIR—Kerem *et al.*[1] recently reported that approximately 70 per cent of the mutations in cystic fibrosis (CF) patients correspond to a specific deletion of 3 base pairs (bp) at amino-acid position 508 (ΔF_{508}) of the putative product of the *CF* gene. To detect this deletion we have developed the two simple polymerase chain reaction (PCR) tests described here.

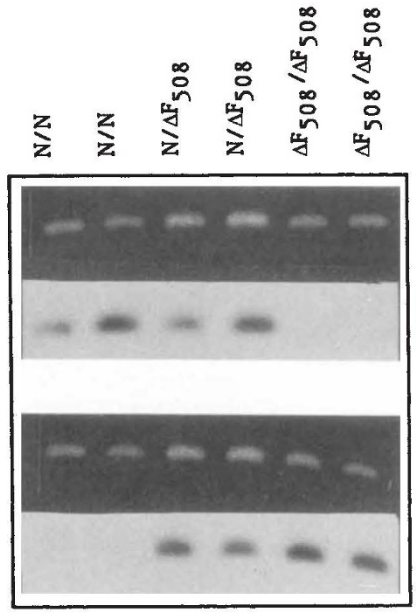

FIG. 1 Ethidium-bromide stained agarose gel electrophoresis of PCR products showing discrimination of normal and ΔF_{508} alleles. N/N, N/ΔF_{508} and ΔF_{508} correspond to homozygous normal, heterozygous and homozygous ΔF_{508} individuals, respectively. In *a*, PCR was performed with primers C16D (ref. 2) and 1468 (5'-GGCACCATTAAAGAAAATATCATC-TT-3'), corresponding to the wild-type allele. In *b*, PCR was performed using primers C16D and 1469 (5'-GGCACCATTAAAGAAAATATCATT-GG-3'), corresponding to the ΔF_{508} deletion allele. The steroid sulphatase (STS) gene primers 908 (5'-GGCCTAGAAGAAGGTTGAAGG-TCCG-3') and 909 (5'-AAGAGGTTGGATGA-GATGGGCATAC-3') were included in each reaction as a positive control for amplification efficiency (292-bp product). PCR (performed according to Saiki *et al.*[3]. Purified genomic DNA (500 ng), 30 pmol of each primer, 2.5 units of Taq polymerase and 2 mM $MgCl_2$ and 200 μM dNTP were used in each 100 μl reaction. After an initial denaturing step at 95 °C for 5 min, 30 cycles were performed, including a 45s denaturing step at 95 °C, an annealing step at 60 °C and a 1 min extension step at 72 °C. The last cycle was followed by a 7 min step at 72 °C. Aliquots of each reaction (15 μl) were electrophoresed on an ethidium bromide stained standard agarose gel (1.3% in Tris-borate buffer) and run for 30 min at 50 mA. The gel was photographed on an ultraviolet transilluminator.

Using the available *CF* cDNA sequence information[2], we designed allele-specific oligonucleotide primers, corresponding to the wild type and F_{508} alleles, and then determined the conditions that enable specific amplification of each of the two alleles by PCR[3,4]. Figure 1 shows the results of allele-specific PCR amplification of DNA from six individuals who had been previously characterized using the allele specific oligonucleotide (ASO) technique (ref. 2; and A. L. Beaudet, personal communication). Two separate reactions were performed for each individual using either the wild-type primer (Fig. 1*a*) or the mutant primer (Fig. 1*b*), in combination with the C16D oligonucleotide[2] as the reverse primer[2].

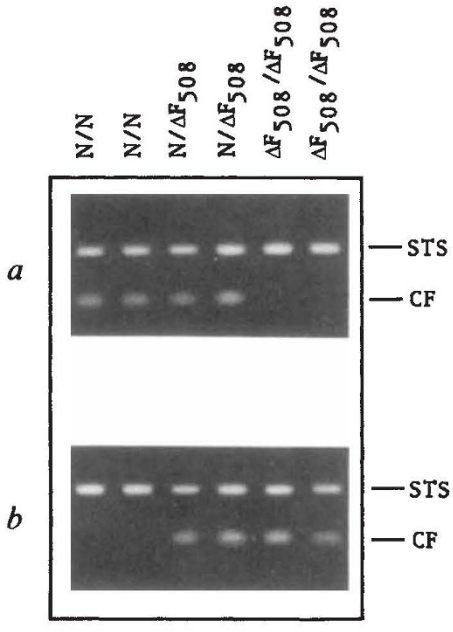

FIG. 2 Simultaneous amplification of normal and ΔF_{508} sequences with both allele specific primers. Reactions contained 30 pmol each of common primer C16D and oligonucleotides corresponding to both normal and ΔF_{508} deletion site. In *a*, normal primer was radiolabelled at the 5' terminus with ^{32}P (~2.5 ci mmol^{-1}). In *b*, mutant primer was radiolabelled. PCR performed as in Fig. 1. PCR products were resolved by ethidium stained agarose gel electrophoresis and are shown above the autoradiograph taken when the gel was dried.

The gel, stained with ethidium bromide, clearly shows that in the lanes corresponding to normal homozygous individuals, amplification of the PCR product of the expected size (79 bp) occurred only when the wild-type primer was present. In the samples from ΔF_{508} deletion homozygotes, the PCR product (76 bp) is visible only in the presence of the mutant primers. In the heterozygotes both the wild-type and the ΔF_{508} primers generated the expected product. As an internal control for the efficiency of amplification, two primers derived from the X-linked steroid sulphatase (STS) gene[5] were included in each reaction.

Figure 2 shows the results obtained when the same primers were used in a competitive oligonucleotide priming reaction[4]. Both allele-specific primers were included in a single PCR and the primer incorporated was identified by the radioactively labelled the 5' terminus. In each case the radioactivity was predominantly incorporated only when the perfectly matched labelled primer was present. The ratio of the radioactive signal from the incorporation of correct-match primer to mismatched primer was approximately 50 to 1. We are at present adapting this to the use of allele-specific fluorescence-tagged primers for single-tube detection[6].

The identification of suitable PCR primer sequences and reaction conditions for the discrimination of ΔF_{508} alleles greatly simplifies PCR diagnosis of CF. It is now possible to identify the genotype of individuals by direct visualization of an ethidium bromide stained gel, without subsequent blotting, ASO hybridization, or washing and exposure.

As the ΔF_{508} deletion is found in the large majority of CF chromosomes (68 per cent)[2] this test may be favoured not only for prenatal and carrier diagnosis in CF families but also for extensive carrier screening in the population.

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A craving for wax

SIR—I have discovered accidentally that our Siamese cat greatly enjoyed the taste of human earwax. I dismissed this as a curiosity until I found that our second Siamese cat also liked it. In fact, the second cat leaps on the bed in the morning hoping to be offered some. I mentioned this odd behaviour to three other people and have learned from them that their cats also liked the wax. I'd always thought that earwax tasted very bitter (as a deterrent to insects getting into your ears), so I find the cats' reaction hard to understand. Does any of your readers have an explanation? I wondered if it might be a means to induce cats to groom their kittens.

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