Polymerase chain reaction for detection of the pMP6d-9/MspI RFLP, a marker closely linked to the cystic fibrosis mutationA.Huth, X.Estivill¹, K.Grade, H.Billwitz, A.Speer, A.Rosenthal, R.Williamson², M.Ramsay² and C.Coutelle

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SOURCE/DESCRIPTION: pMP6d-9 is a human genomic clone with a 2.6kb HindIII insert in pBR322(1). HindIII/MspI subclones were sequenced in order to obtain oligonucleotides suitable for detection of the MspI RFLP within pMP6d-9 by PCR.

POLYMORPHISM: With Southern blotting the MspI RFLP has alleles of 13kb(allele 1) and 8.5 + 4.5kb(allele 2). Allele 1 represents the absence of the MspI site and allele 2 the presence of the site.

FREQUENCY: Determined by Southern blotting in four European Caucasoid populations(1).

	CF chromosomes (n=134)	non-CF chromosomes (n=134)
allele 1 :	0.13	0.65
allele 2 :	0.87	0.35

NOT POLYMORPHIC FOR: Not known

CHROMOSOMAL LOCALISATION: pMP6d-9 is physically as well as genetically close to the CF locus which maps to chromosome 7q31(1).

MENDELIAN INHERITANCE: Codominant Mendelian inheritance has been illustrated in several hundred families with Southern blotting and in more than one hundred with PCR.

PROBE AVAILABILITY: Not applicable

OTHER COMMENTS: The PCR oligonucleotide pair 5'AATGCAACAATTACCCCAATTGCTCA3' and 5'GGTTAGGTGAGAGAACAAAGCAAATT3' produce a 377bp sequence containing the polymorphic MspI site. In the presence of the site, digestion with MspI gives a 204bp and a 173bp fragment. The figure illustrates the RFLP in two nuclear families after amplification using 1.5U of Taq-polymerase (Cetus/Perkin-Elmer), 0.5 ug genomic DNA and 50 nM of each oligonucleotide. Initial denaturation for 5 min in boiling water, followed by 30 cycles of 1 min, 95°; 0.5 min, 47°; 1 min, 73°.

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