PCR detection of the pKM.19/ScrfI RFLP (D7S23), a marker closely linked to the cystic fibrosis mutation

V.Nunes*, M.Chillón, N.Lench¹, M.Ramsay¹ and X.Estivill

Molecular Genetics Department, Fundació d'Investigació, Sant Pau, 08025 Barcelona, Spain and ¹Molecular Genetics Department, St Mary's Hospital Medical School, London W2 1PG, UK

Source/Description: pKM-19 is a 1.0 kb EcoRI human genomic fragment inserted in pUC13, that detects a Scrfl (CC/NGG) RFLP (1, 2). We report here the primer sequences suitable for the detection of this RFLP by PCR.

Polymorphism: Scrfl digest of the amplified fragments identifies two alleles: 412 bp (A1) and 212 + 200 bp (A2). Allele 1 represents the absence of the Scrfl site, and allele 2 the presence of the site (Figure).

Frequency: European Caucasoid Population from 320 unrelated individuals: CF (A1) = 0.67, (A2) = 0.33; non-CF (A1) = 0.04, (A2) = 0.96.

Chromosomal Localization: The polymorphic Scrfl recognition site maps approximately at 200 kb from the CFTR gene on 7q31 (ref. 3).

Mendelian Inheritance: Co-dominant segregation demonstrated in 98 families.

Other Comments: The oligonucleotide sequences for PCR are: MIV1: 5'CCTTCTAGGCTGTGTGGCT3' and VIM2: 5'GTGGCTCAGAGATTCTGCC3'. We carry out PCR as recommended by the supplier, using 400 ng of genomic DNA, 90 pmoles of each primer, 200 μ M dNTPs, and 2 units of Taq polymerase (Cetus/Perkin Elmer). Initial denaturation for 5 min followed by 35 cycles of 15 sec 95°C, 45 sec 60°C, and 30 sec 74°C. The amplified products were digested overnight with Scrfl. DNA fragments are resolved by electrophoresis through a 2% agarose gel.

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Tetranucleotide repeat polymorphism in the vWF gene

Hans K.Ploos van Amstel and Pieter H.Reitsma

Haemostasis and Thrombosis Research Unit, University Hospital, PO Box 9600, 2300 RC Leiden, The Netherlands

Source/Description: Two primers (vWFII-5 TGTACCTAGTTA-TCTATCCTG and vWFII-3 GTGATGATGATGGAGACAG-AG) were used to amplify a 166 bp long TCTA repeat rich region (nt 2215-2380) of intron 40 of the human vWF gene (1).

Polymorphism: The PCR dependent DNA amplification with vWFII-5 and vWFII-3 detects six alleles designated WBII1-6 in decreasing order of size (see figure) 174, 170, 166, 162, 158 and 154 bp respectively.

Frequency: A1, 0.08; A2, 0.06; A3, 0.27; A4, 0.35; A5, 0.05; A6, 0.19 as estimated from 24 unrelated caucasians.

Chromosomal Localization: The vWF gene has been localized on chromosome 12 (2).

Mendelian Inheritance: Co-dominant inheritance.

Availability: Contact P.H.Reitsma.

Comments: The WBII alleles are not in linkage disequilibrium with the alleles found for the TCTA repeat rich region located upstream (nt 1900-2000) in the vWF gene (3). The conditions of the polymerase chain reactions were essentially as described (4) consisting of 35 cycles with denaturing at 94°C for 1 min annealing at 55°C for 1.5 min and extension at 71°C for 3 min. The fragments were visualized on a 0.4 mm thick 6% PAGE-gel by staining with ethidium bromide.

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* To whom correspondence should be addressed