Dinucleotide repeat polymorphism closely linked to the cystic fibrosis (CFTR) gene

B.Richards, C.Reeves and G.T.Horn

Department of Genetic Disease Research, Integrated Genetics, Framingham, MA 01701, USA

Source/Description: A polymorphic region consisting of repeats of the dinucleotide (GT), was identified with a (CA)₁₀ oligonucleotide probe in a cosmid (1) containing the KM.19 and CS.7 probe fragments near the CFTR gene (2). A fragment containing the polymorphic region, and lying approximately 3 kb centromeric to the CS.7 probe fragment (3), was cloned and sequenced (EMBL # X60680) to reveal the exact nature of the repeat and its flanking sequence.

Primer Sequences:

GH425: 5'-GACGTGCTAGCCTGGTCTCCAGCTCT-3'

GH426: 5'-GATGGGGGAGGCGGTTGTAGTTTT-CAAGGT-3'

Allele Frequencies: Nine different allelic products were observed among 66 chromosomes from unrelated Caucasians (heterozygosity = 82%).

Allele (bp)	Frequency	Allele (bp)	Frequency
G9 109	0.03	G4 119	0.12
G8 111	0.06	G3 121	0.20
G7 113	0.35	G2 123	0.06
G6 115	0.04	G1 127	0.03
G5 117	0.11		

Mendelian Inheritance: Codominant segregation was observed in six families.

Chromosomal Location: The KM.19 and CS.7 loci map to D7S23, very close to the CFTR gene.

Comments: General amplification conditions were previously described (4), but we used 0.4 μ M of each primer (GH425 and GH 426) plus 10 nM γ -³²P-ATP kinased GH425. Samples were amplified for 22 cycles at 94°C for 10s, 60°C for 10s, and 74°C for 10s, with a final incubation at 74°C for 5 m. PCR products were analyzed on a 6% denaturing polyacrylamide sequencing gel run at 60W for 2.5 hours. The 109 bp allele contains 19 (GT) repeats. Some individuals from CF families were also analyzed. Among ten chromosomes harboring the Δ F508 mutation, six contained the 121 bp allele, three the 123 bp allele, and one the 119 bp allele. The Δ F508 mutation has also been found by others to be associated with more than one allele of a polymorphic dinucleotide repeat region within the CFTR gene (5).

Acknowledgements: We would like to thank Linda Lopez for additional sequencing, and Greg Landes and Gabriela Wright for isolation and mapping of D7S23 cosmids.

References: 1) Horn *et al.* (1990) *Clin. Chem.* **36**, 1614–1619. 2) Estivill *et al.* (1987) *Nature* **326**, 840–845. 3) Rommens *et al.* (1989) *Science* **245**, 1059–1065. 4) Saiki *et al.* (1988) *Science* **239**, 487–491. 5) Morral *et al.* (1991) *Genomics* **10**, 692–698.

BanI RFLP at AK1 locus (9q34)

D.E.Schuback¹, L.Ozelius^{1, 2} and X.O.Breakefield^{1, 3,*}

¹Neuroscience Center, Neurology Department, Massachusetts General Hospital-East, Charlestown, MA 02129, ²Genetics Department and ³Neuroscience Program, Harvard Medical School, Boston, MA 02115, USA

Source/Description: (hAK1B3.25) is a 3.25 kb BamHI fragment spanning exons 2–5 of the adenylate kinase-1 gene (1).

Polymorphism: BanI digestion gives a two allele polymorphism with bands at 2.2 kb and 1.6 kb along with a constant band at 1.7 kb.

Frequency: Among 78 unrelated North Americans.

Allele	Frequency	Size
D1	0.76	2.2 kb
D2	0.24	1.6 kb

The observed heterozygosity is 0.36

Not Polymorphic For: EcoRI, BamHI, HindIII, BglII, PstI, SacI, XmnI, DraI, EcoRV, StuI, BclI, StyI, RsaI, MspI.

Chromosomal Localization: This probe has been localized to 9q34 (2).

Mendelian Inheritance: Co-dominant segregation pattern has been observed in 12 families (176 individuals).

Probe Availability: Contact T. Bech-Hansen.

Other Comments: TaqI polymorphism has previously been reported (1).

Acknowledgements: Supported by Dystonia Medical Research Foundation and NIH grant NS28384.

References: 1) Bech-Hansen *et al.* (1989) *Nucl. Acids Res.* **17**, 4004. 2) Ferguson-Smith *et al.* (1976) *Hum. Genet.* **34**, 35–43.

* To whom correspondence should be addressed