Polymerase chain reaction (PCR) for detection of ApaI polymorphism at the insulin like growth factor II gene (IGF2)

K.Tadokoro[1], H.Fujii[1,2], T.Inoue[2] and M.Yamada[1]*
[1]National Children's Medical Research Center, 3-35-31, Taishido, Setagaya, Tokyo 154 and [2]Laboratory of Nucleic Acid Science, College of Agriculture and Veterinary Medicine, Nihon University, Fujisawa, Kanagawa 252, Japan

Source/Description: A cDNA clone of the human insulin-like growth factor II (IGF2) detects ApaI polymorphism (1), which has been registered in the polymorphic data of Human Gene Mapping 10 as System D of the IGF2 gene. The polymorphic ApaI site was deduced to be at the 8763−8768th nucleotide in a reported sequence (2) (GenBank X03562). PCR primers were designed to amplify a 236 bp fragment containing the polymorphic site, contained within non-coding sequence of exon 4 (3'UTR).

Primer Sequences:
IGF2F 5'-CTTGGACTTTGAGTCAAATTGG (8703−8724 bp in X03562)
IGF2R 5'-CCTCCTTTGGTCTTACTGGG (993−974 bp in X07868)

Polymorphisms: ApaI digestion of the PCR product of 236 bp (D1) generates two fragments of 63 and 173 bp (D2) if the site is present. Direct sequencing of the PCR products revealed a transition at the 8765th nucleotide, forming GGACCC in D1 but GGGCCC in D2.

Frequency: Studied in 65 unrelated individuals of the normal Japanese.
D1 0.47
D2 0.53
Observed heterozygosity was 57%, expected, 50%.

Mendelian Inheritance: Co-dominant segregation was confirmed in 15 two-generation families (3).

Chromosomal Location: IGF2 gene has been localized to 11p15.5.

Other Comments: Amplification was carried out in 30 cycles of 1 min at 92°C, 1 min at 55°C and 2 min at 72°C using a program control temperature system (PC-700 Astec). The PCR products were digested with ApaI after 2-fold dilution with 10 mM Tris pH 7.4 with 20 mM $MgCl_2$ and then analyzed by electrophoresis through a 2% NuSieve 3:1 gel in TBE buffer.

References: 1) Xiang,K., Cox,N.J. and Bell,G.I. (1988) *Nucl. Acids Res.* **16**, 3599. 2) Dull,T.J., Gray,A., Hayflick,J.S. and Ullrich,A. (1984) *Nature* **310**, 777−781. 3) Tadokoro,K., Kato,M., Yokota,S. and Yamada,M. (1991) *Nucl. Acids Res.* **19**, 2514.

* To whom correspondence should be addressed

Dinucleotide repeat polymorphism in the human SRC gene on chromosome 20

K.Xiang, G.Phillippe, M.Seino, K.Bonham[1], D.J.Fugita[1] and G.I.Bell*
Howard Hughes Medical Institute, University of Chicago, 5841 S. Maryland Avenue, Box 391, Chicago, IL 60637, USA and [1]Cell Regulation Group, Department of Medical Biochemistry, University of Calgary Medical Centre, Calgary, Alberta T2N 4N1, Canada

Primers/Description:
Two primers (src11B-A, 5'-TTCAAGTGGTTGCCTCTGGC-3', and src11B-B, 5'-AGCAACTTGCCCAGGCTATGA-3') were used to amplify a 191−207 bp CA-repeat rich region in the human SRC gene.

Frequency: Nine alleles were observed in 31 unrelated Caucasians. The heterozygosity was 71%.

Allele	bp	Frequency	Allele	bp	Frequency
A1	207	0.03	A2	205	0.03
A3	203	0.04	A4	201	0.10
A5	199	0.10	A6	197	0.37
A7	195	0.29	A8	193	0.02
A9	191	0.02			

Chromosomal Localization: SRC was assigned to chromosome 20q11.2 (1).

Mendelian Inheritance: Co-dominant inheritance was observed in a five-generation pedigree with maturity onset diabetes of the young in which 87 members were typed (2).

Other Comments: The PCR was performed using ^{32}P-labeled src11B-A and unlabeled src11B-B for 30 cycles: denaturation at 94°C for 1 min; annealing at 65°C for 1 min; and extension at 72°C for 2 min. The PCR products were analyzed on a 5% denaturing polyacrylamide gel (Figure 1). The dinucleotide repeat sequence was of the form $(CA)_{20}$ and was identified in the human SRC cosmid Cos 11B; the complete sequence of the region around this repeat is available from the authors.

References: 1) Grzeschik,K.H. and Skolnick,M.H. (1990) *Cytogenet. Cell Genet.* **55**, 229. 2) Bell,G.I. *et al.* (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1484.

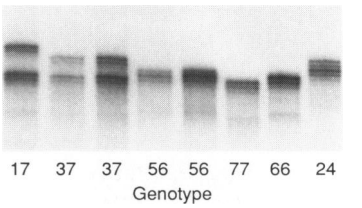

Figure 1. PCR amplification of CA-repeat in the human SRC gene. The genotypes are noted at the bottom of the figure.

* To whom correspondence should be addressed