An STS in the human IL7 gene located at 8q12–13

Laurie L.Brunton and Stephen D.Lupton*
Immunex Corporation, 51 University Street, Seattle, WA 98101–2977, USA

Submitted December 18, 1989

The DNA sequence and chromosomal location of the human interleukin-7 (IL7) gene have been reported (1, 2, 3). We have combined this information in a sequence-tagged site (STS), designated hIL-7.1/8q12–13, for inclusion in the human genome map (4). Using the polymerase chain reaction (PCR) described below, a fragment of the expected size (322-bp) was amplified from human genomic DNA. The fragment contained sequences from the 3′ end of the human IL7 gene (3754–4075 in ref. 2), located at 8q12–13 (ref. 3). The amplified fragment was purified, uniformly radiolabelled, and used to probe a human genomic Southern. As anticipated (2), a single HindIII fragment of ~5-kbp was detected, demonstrating both the unique character of the amplified sequences, and the direct utility of the PCR product as a probe for retrieving clones containing this STS from libraries.

PCR primers: Forward (3754–3773 in ref. 2)
CATACAGCATTACAAATTGC
Reverse (4075–4059 in ref. 2)
TGTAGATTCTGGCCTGC

PCR components: 100 ng of human genomic DNA, 1 μg of each oligonucleotide, 200 μM dNTPs, and 2.5 U Taq polymerase (Perkin Elmer Cetus) in 100 μl of 1×PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3 (at room temperature), 1.5 mM $MgCl_2$, 0.1% (w/v) gelatin).

PCR profile:
94°C for 2 minutes
50°C for 2 minutes
72°C for 1 minute — for 30 cycles.
94°C for 1 minute
72°C for 5 minutes

Anticipated sequence of the PCR product:

```
CATACAGCATTACAAATTGCTTACTTTGGAATACATTTCTCCTTTGATAAAATAAATG
AGCTATGTATTAACACTGCCAGATTCAGTTAATAAATCTCAACAGAATTTTTAAGGT
GAGATTTTTAATACTTCACTGCTCTTTAATTTTCTACTTTCATTGAATATATGACCTTT
AATAGCCTATTACCAACAATGAATATGAGACATTACCTTCTATGTTAAATTTTAGTCT
TTTAAAAGAAACTTGTCTGGAAATGTCTGCAGTCTTCATTCACTGATAAAGGGAAAT
GGAAACACTATTCTAAGCAGGCCAGAATCTACA
```

References: 1. Goodwin,R.G. *et al*. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 302. 2. Lupton,S.D. *et al*. (1990) *J. Immunol.* in press. 3. Sutherland,G.R. *et al*. (1989) *Hum. Gen.* **82**, 371. 4. Olson,M. *et al*. (1989) *Science* **245**, 1434.

A MaeIII polymorphism near the dystrophin gene promoter by restriction of amplified DNA

R.G.Roberts, M.Bobrow and D.R.Bentley
Paediatric Research Unit, Guy's Hospital, London SE1 9RT, UK

Source/Description: A search for polymorphisms within a 1.4kb portion of pERT84[1] (DXS142) using amplification and mismatch detection (AMD) analysis[2] followed by direct sequencing demonstrated the presence of a polymorphic MaeIII restriction site. The polymorphism is readily detected by performing a polymerase chain reaction[3] (PCR) with the oligonucleotides 5′-CAGGGATGCAAAGGAACTGGG-3′ and 5′-CAGTTTGTTTAACAGTCACTC-3′, and digesting the 252bp product with MaeIII (Boehringer Mannheim).

Polymorphism: The sequence change detected is a CpG-CpA transition within the recognition sites for both MaeIII and HphI. The MaeIII digestion products are analysed on 5% polyacrylamide minigels (see figure). Digestion should yield 236bp plus 16bp for the 841Q− form (lane 5), and 128bp plus 108bp plus 16bp for the 841Q+ form (lanes 2–4).

Frequency: Studied in 78 unrelated females.

841Q−	0.74
841Q+	0.26

Chromosomal Localisation: Xp21.2, within 30kb of the promoter region of the dystrophin gene.

Mendelian Inheritance: Co-dominant X-linked segregation was observed in 4 families.

Other Comments: PCR was performed using 30 cycles of 1′ at 93°, 1′ at 62°, 2.5′ at 72°. This RFLP particularly useful for defining the position of crossovers detected by flanking markers, and in conjunction with MP1P[2] for obtaining an estimate of the recombination rate across the dystrophin gene.

References: 1)Kunkel,L.M., Monaco,A.P., Middlesworth,W. *et al*. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4778–4782. 2) Roberts,R.G., Montandon,A.J., Bobrow,M. and Bentley,D.R. (1989) *Nucl. Acids Res.* **17**, 5961. 3) Saiki,R.K., Gelfand,D.J., Stoffel,S. *et al*. (1988) *Science* **239**, 487–491.

Acknowledgements: This work was supported by the Medical Research Council, the Generation Trust, and the Muscular Dystrophy Group of Great Britain and Northern Ireland.