A dinucleotide repeat polymorphism at the D9S109 locus

R.A.Furlong, J.E.W.Lyall, D.R.Goudie, M.A.Leversha, N.A.Affara and M.A.Ferguson-Smith

Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK

Source/Description: Clones containing [CA]_n microsatellites were selected with end-labelled [TG]₁₅ from a flow-sorted chromosome 9 library (derived from a human/hamster hybrid cell line) in Charon 21A. Southern blots of clones containing microsatellites were probed with either human or hamster DNA. Clone 9CMP1 containing a [CA]_n microsatellite of human origin was sequenced enabling the design of PCR primers (Yuille *et al.*, 1991). Microsatellite sequence showed the repeat to be [CA]₁₁.

Primer Sequences:

5' GCA CAG GCT GCA ATA TAG AC 3' (CA strand)

5' TTT ACT GTA TAA AAA CTG AAG CTA ATA 3' (GT strand)

Polymorphism: Heterozygosity was estimated at 70% by analysis of 196 chromosomes from unrelated individuals.

Allele	bp	Frequency
A1	229	0.038
A2	227	0.258
A3	225	0.279
A4	223	0.100
A5	221	0.167
A6	219	0.158

Chromosomal Localization: 9CMP1 was assigned to 9q22.1–9q32 by PCR across a series of flow-sorted chromosome 9 translocations (Cotter *et al.*, 1989) and to 9q31 by *in situ* hybridization. 2 point linkage analysis of 9CMP1 with LAMP 92 (Pandolfo *et al.*, 1988) produced a recombination fraction of 0.03 with a maximum LOD score of 10.95; and with 9CMP2 (Lyall *et al.*, 1991), a recombination fraction of 0.01 with a maximum LOD score of 8.39.

Mendelian Inheritance: Co-dominant segregation was observed in 19 CEPH families of 2 and 3 generations.

PCR Conditions: 50–100 ng genomic DNA were amplified in a 10 µl reaction containing: 125 µM dATP, dGTP and dTTP; 25 µM cCTP, 1 µCi α³²P-[dCTP], 25 mM TAPS pH 9.3, 50 mM KCl, 1 mM DTT, 2 mM MgCl₂, 0.05% W1 detergent, 50 ng of each primer and 0.75 units *Taq* polymerase (NBS). Amplification conditions were as follows: 94°C for 4.5 min; 30 cycles of 94°C for 0.5 min, 55°C for 0.5 min, 72°C for 0.5 min; and a final extension at 72°C for 10 min.

Acknowledgements: This work was supported by the MRC Human Genome Mapping Project, and the ICRF.

References: 1) Yuille, M.A.R. *et al.* (1991) *Nucleic Acids Res.* **19**, 1950. 2) Cotter, F. *et al.* (1989) *Genomics* **5**, 470–474. 3) Pandolfo, M. *et al.* (1988) *Nucleic Acids Res.* **16**, 7213. 4) Lyall, J.E.W. *et al.* (1992) *Nucleic Acids Res.* **20**, 925.

A dinucleotide repeat polymorphism at the D9S127 locus

J.E.W.Lyall, R.A.Furlong, M.A.R.Yuille, D.R.Goudie, M.A.Leversha, N.A.Affara and M.A.Ferguson-Smith

Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK

Source/Description: Clones containing [CA]_n microsatellites were selected with end-labelled [TG]₁₅ from a flow-sorted chromosome 9 library (derived from a human/hamster hybrid cell line) in Charon 21A. Southern blots of clones containing microsatellites were probed with either total human or hamster DNA. Clone 9CMP2 was shown to contain a [CA]_n microsatellite of human origin by this process. Direct sequencing out of this [CA]_n repeat enabled PCR primers to be designed (Yuille *et al.*, 1991). By sequencing with one of the PCR primers, the microsatellite was shown to contain 16 CA repeats, in this clone.

Primer Sequences:

5' CCC TCA AAA TTG CTG TCT AT 3' (CA strand)

5' AGA TTG ATT GAT ACA AGG ATT TG 3' (GT strand)

Polymorphism: Heterozygosity was estimated at 72% by analysis of 210 chromosomes from unrelated individuals.

Allele	bp	Frequency
A1	159	0.062
A2	157	0.447
A3	155	0.034
A4	153	0.284
A5	151	0.067
A6	149	0.106

Chromosomal Localization: 9CMP2 was assigned to 9q22.1–9q32 by PCR across a series of flow-sorted chromosome 9 translocations and to 9q31 by *in situ* hybridization. Linkage analysis in CEPH families indicated that 9CMP2 and the LAMP 92 locus (Pandolfo *et al.* 1988) were linked with a peak LOD score of 8.27 at a recombination frequency of 0.04.

Mendelian Inheritance: Co-dominant segregation was observed in 31 families of 2, 3, and 4 generations.

PCR Conditions: 50–100 ng genomic DNA were amplified in a 10 µl reaction containing: 125 µM dATP, dGTP, dTTP, 25 µM dCTP, 1 µCi α³²P-dCTP, 25 mM TAPS pH 9.3, 50 mM KCl, 1 mM DTT, 2 mM MgCl₂, 0.05% W1 detergent, 50 ng of each primer and 0.75 units *Taq* polymerase (NBS). Amplification conditions were as follows: 94°C for 4.5 min, 30 cycles of 94°C for 0.5 min, 55°C for 0.5 min, 72°C for 0.5 min and a final extension at 72°C for 10 min.

Acknowledgements: We thank Dr Joe W.Gray (formerly of the Lawrence Livermore National Laboratory, Berkley, CA) for the Chromosome 9 library. This work was supported by the Medical Research Council Human Genome Mapping Project, and the Imperial Cancer Research Fund.

References: 1) Yuille, M.A.R. *et al.* (1991) *Nucleic Acids Res.* **19**, 1950. 2) Pandolfo, M. *et al.* (1988) *Nucleic Acids Res.* **16**, 7213. 3) Cotter, F. *et al.* (1989) *Genomics* **5**, 470–474.