Dinucleotide repeat polymorphism within ERCC5 gene

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Source/Description: A human genomic DNA library in EMBL3 was screened with a cDNA probe encoding the conserved I-region of ERCC5 (1), corrects the DNA repair defects in both xeroderma pigmentosum complementation group G and rodent ERCC group 5 (1–3). A 2.6 kb *Eco*RI fragment of one positive phage, phage #10, was found to contain a $(CA)_{19}$ repeat. The repeat and surrounding sequences (EMBL accession no. X75341) lie within an intron that disrupts the codon for Gly652.

PCR Primers:

CA strand: 5'-GCAATGACTCGGTATTGG-3'
GT strand: 5'-TGGATGCTAACAAGTGGG-3'

Polymorphism:

Allele	bp	Frequency	Allele	bp	Frequency
A1	277	.01	A7	265	.06
A2	275	.05	A8	263	.14
A3	273	.13	A9	261	.34
A4	271	.04	A10	257	.04
A5	269	.03	A11	245	.01
A6	267	.15			

Heterozygosity = 0.84 observed in 121 unrelated individuals in the CEPH reference pedigrees.

Allele Identification in Selected CEPH Individuals:

13291-01:	A3/A9	1333-01:	A9/A11	1331-02:	A7/A8
1333-01:	A9/A11	13291-02:	A4/A10	1333-02:	A2/A7

Mendelian Inheritance: Observed in all 40 CEPH pedigrees.

Chromosomal Localization: 13q33 by FISH.

Mapping by Linkage Analyses: Two-point linkage analysis in CEPH families showed no recombination with D13S158 (θ = 0.00; lod = 25.59). Multipoint analysis placed XPG between D13S122 and COL4A1 with odds > 1000:1.

PCR Conditions: The 22 μl reactions contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 50 μM of each dNTP, 400 ng of genomic DNA, 0.75 pmoles of CA strand primer 5'-end labelled with [γ-^{32}P]ATP by T4 polynucleotide kinase, 10 pmoles of CA strand primer, 20 pmoles of GT strand primer, and 1 unit of Taq polymerase (Perkin-Elmer/Cetus). Samples were heated for 7 min at 94°C then subjected to 35 cycles of 30 sec at 94°C, 30 sec at 51°C, and 45 sec at 72°C. Amplified products were electrophoresed through 6% denaturing polyacrylamide gels.

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Tetranucleotide repeat polymorphism at the D8S346 locus

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Source/Description: The polymorphic (A_3T) repeat KW400 was isolated from a *Sau* 3A subclone of a flow-sorted chromosome 8 specific cosmid library (LA08NC01) (1) by hybridization to an $(A_3T)_{20}$ oligomer. The predicted length of the amplified sequence is approximately 240 bp (GenBank accession number L12264).

PCR Primers:

KW400A 5'-TGC ATG ACA GCC TGG GTG ACA GAA
KW400B 5'-CCA CAG GTG CCC ACG CTA AGA CA

Frequency: Estimated from 200 chromosomes of unrelated Caucasian individuals.
Observed Heterozygosity Index = 63%.

Allele	bp	Frequency	Allele	bp	Frequency
A1	252	0.005	A4	240	0.515
A2	248	0.025	A5	236	0.380
A3	244	0.065	A6	232	0.010

CEPH 884.1—5,5 884.2—3,4

Mendelian Inheritance: Co-dominant segregation was observed in 5 informative Caucasian familes.

Chromosomal Localization: KW400 has been assigned to chromosome 8q (Region I) by PCR of a somatic cell hybrid mapping panel (2).

PCR Conditions: PCR is performed in 25 μl containing 200 ng DNA, 0.5 μM of each primer, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.4), 40 mM NaCl, 200 μM of each dNTP, 0.25 mM spermidine HCl, 0.625 units of Taq polymerase (Ampli-Taq, Perkin-Elmer/Cetus), and 0.01 μM of radiolabelled KW400B (5' end-labelled with γ-^{32}P ATP (3,000 Ci/mmol) using 30 μCi/10 pmol primer). Initial denaturation was for 5 minutes at 94°C. Amplification was for 30 cycles with denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 60 seconds. The products were separated by electrophoresis on a 32% formamide, 5.7 M urea, 7% polyacrylamide denaturing gel and analysed by autoradiography.

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