JCB
Online Supplemental Material

Engelholm et al. http://www.jcb.org/cgi/doi/10.1083/jcb.200211091

Construction of targeting vector and generation of transgenic mice

A mouse genomic 129/SvJ bacteriophage lambda DNA library was screened by in situ hybridization with a 319-bp ^{32}P-labeled cDNA fragment generated by RT-PCR from murine fibroblast RNA using the primers uPARAP/Endo180-fw (5′-CTGCGCTGCGTCTTGCTTCT-3′) and uPARAP/Endo180-rv (5′-CTGCCATCCGAAGACTCAAG-3′) corresponding to nucleotides 150–169 and 449–468, respectively, of the murine uPARAP/Endo180 cDNA sequence (GenBank/EMBL/DDBJ accession no. U56734). The targeting vector was generated from a purified phage clone containing a 17-kb insert that included the putative intron 2 through the putative exon 9. This fragment was subcloned into pBluescript® II (Stratagene) and characterized extensively by restriction mapping, Southern blotting, and sequencing of the intron/exon boundaries. The targeting vector was constructed by inserting a Herpes simplex virus–thymidine kinase expression cassette into the RsrII site of a Scrambler 924 gene-targeting vector (Stratagene) to provide a means of selection against random insertion of the targeting vector. This step was followed by simultaneous insertion of a 4-kb BamHI-EcoRI fragment that included the putative exon 7 through the putative exon 9, and a PCR-generated XhoI-BglII bovine growth hormone polyadenylation signal between the XhoI and EcoRI sites of the vector. Thereafter, an 835-bp fragment from intron 1 that was subcloned into a pBluescript® SK+ vector was inserted between the NotI and HindIII sites. Finally, a phosphoglycerate kinase–hypoxanthine phosphoribosyl transferase (HPRT) cassette was inserted into the AscI site to generate the completed targeting vector. The targeting vector was introduced into HM-1 embryonic stem (ES) cells (Magin, T.M., J. McWhir, and D.W. Melton. 1992. *Nucleic Acids Res*. 20:3795–3796) by electroporation, and ES cell clones were selected in HAT medium (GIBCO BRL) including 2 μM ganciclovir (Syntex). Resistant ES cell clones were isolated, expanded, and screened for homologous recombination of the targeting vector into the uPARAP/Endo180 locus by PCR using the primer HPRT#1 (5′-TATTACCAGTGAATCTTTGTCAGCAG-3′), complementary to the 3′ end of the PGK-HPRT minigene, and the primer uPARAPG1209 (5′-GGAGTGCTAGTACCTCGCTGTTGTAGT-3′), complementary to a sequence of the uPARAP/Endo180 gene that is located upstream of the short arm of the targeting vector. uPARAP/Endo180 gene targeting was further verified by Southern blot hybridization of BamHI-digested genomic DNA using a ^{32}P-labeled 554-bp probe that was external to the targeting vector sequences. Two independently targeted ES cell clones were injected into the blastocoel cavity of C57Bl/6J-derived blastocysts and implanted into pseudopregnant females. Chimeric male offspring was bred to NIH Black Swiss females (Taconic Farms) to generate heterozygous offspring derived from both ES cell clones. These mice were subsequently interbred to generate homozygous uPARAP/Endo180$^{-/-}$ progeny. Genotyping of mice was performed by Southern hybridization as described above, or by PCR amplification of DNA from ear or tail biopsies with the primers uPARAPtarg (5′-TCCTACAAATACACGCTGGCGATA-3′) and HPRT (5′-GCAGTTCCCTTTTAAATGCAAATCA-3′), to detect the presence of the targeted uPARAP/Endo180 allele, and uPARAP/Endo180 E3–3′ (5′-TCTACACCATCCAGGGAAACTCAC-3′) and uPARAP E3−5′ (5′-TTAAACTGG-TAACAGCTGTCAGTC-3′), to detect the wild-type uPARAP/Endo180 allele.