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#### Construction of targeting vectors and analysis of recombinant clones.

Amplification of the three POLH genomic fragments was performed from genomic DNA of the ES cell line E14.1 with the following primers: fragment 5' (nested PCR, 3 kb final): first PCR, 5'-5'-1, 5'-ATGTCCAGATCTTCCTGG-3' and 5'-3'-1, 5'-CAGTCGACTTCGTTGCCCTTCCAG-3' (Sall site underlined) (10 s at 92°C, 30 s at 58°C, 8 min at 68°C, 15 cycles with Pfu Turbo); second PCR: 5'-5'-2, 5'-CACTCGAGATGACTTGGTTGAGGAGGTG-3' (XhoI site underlined) and 3'-1 (same conditions, 35 cycles); fragment exon 4 (780 bp): exon4-5', 5'-CAGAGACTGCTGCAGAATGC-3' and exon4-3', 5'-TCAGTCTCCACCTTCCACTG-3' (30 s at 94°C, 30 s at 60°C, 1 min at 72°C, 40 cycles, with Pfu Turbo); and fragment 3' (3 kb): 3'-5', 5'-CACTCGAGGTGATTGCTTCATCTGCAGG-3' and 3'-3', 5'-CACTCGAGCAACTCA-GAGATCCACCTGC-3' (XhoI site underlined) (10 cycles with Long Expand PCR System [Boehringer] with a 60°C annealing, 30 cycles with Pfu Turbo, 30 s at 94°C, 30 s at 58°C, 8 min 72°C). Fragments 3' and 5' were first cloned in pCR-XL-TOPO vector (Invitrogen), excised by Sall or XhoI digestion, and inserted in the Sall or XhoI site of the pFlox vector. Exon 4 was cloned directly in the BamHI site of this vector.

Homologous recombination was diagnosed by PCR on both sides of the putative insertion: 5' screening with primers 5'-5'-1 and exon 4-3' (37 cycles, 68°C annealing, Long Expand PCR System). PCR products were analyzed by Southern blotting, using a loxP-specific oligonucleotide probe, 5'-GCTGGACGTAAACTCCTCTTCAGAC-3', and loxP-positive products were sequenced. 3' screening was performed with 5'-AGCCTTGTTCCGAATAGCC-3' (within *Polh* exon 5) and 5'-CCTCTGAAAACCACACTGC-3' (within thymidine kinase; same PCR conditions). Excision of the loxP-flanked neoR-thymidine kinase genes was diagnosed by the same PCR used to screen tail DNA from targeted mice.