

GK Gene Targeting Vector

The gene targeting vector used in Postic et al, 1999 was assembled as follows. 1. pLP72 was created by removing the *loxP* sequence from pBS246 (Sauer, 1993) with EcoRI and HinD III and inserting it into the corresponding sites of pSP72 (Promega). 2. A ~5 kb Sal I-BamH I fragment of the mouse glucokinase gene was removed from λ 21 (Postic et al, 1995) and cloned into the Sal I and BamH I sites of pSL1180 (Pharmacia). 3. The *loxP* sequence in LP72 was removed for a second time by digestion with Bgl II and BamH I and cloned into the BamH I site of the plasmid from step 2. 4. A ~4 kb BamH I fragment of the mouse glucokinase gene from λ 21 was subcloned into pBluescript (Stratagene). 5. The Sal I and Xho I sites in the plasmid from step 4 were destroyed by Klenow fill-in. 6. The Xmn I site in the plasmid from step 5 was converted to an Xho I site by linker insertion. 7. The BamH I site in LP72 was destroyed by Klenow fill-in. 8. The *loxP* sequence in the plasmid from step 7 was removed by digestion with Cla I and HinD III and cloned into the corresponding sites of pNTK(B) (gift from Dr. Richard Mortensen, Harvard Medical School). 9. The insert from the plasmid in step 8 (containing a *loxP* site and pgk-neo) was removed as a Sal I fragment and cloned into the Xho I site in the plasmid from step 6. 10. The insert in the plasmid from step 9 was removed by digestion with BamH I and cloned into the BamH I and Bgl II sites of the plasmid from step 9. 11. The TK cassette from pNTK(A) was removed as a Not I-SpeI fragment and inserted into the corresponding sites of the plasmid from step 10.

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B. Sauer, *Methods Enzymol.* 225, 890-900, 1993.

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