

Sox10-iCreER(T2)

Category
Biological Materials/Genetically
Modified Organisms

Sox10-iCreER(T2): Codon-improved version of CreER(T2) expressed under the transcriptional control of Sox10 sequences in a PAC.

Sox10-iCreER^{T2}. Codon-improved version of CreER^{T2} expressed under the transcriptional control of Sox10 sequences in a PAC. This is designed to express in myelinating cells of the CNS and PNS (oligodendrocytes and Schwann cells, respectively) following tamoxifen administration. Pre-natal tamoxifen might also induce expression in migratory neural crest cells, although this has not been tested.

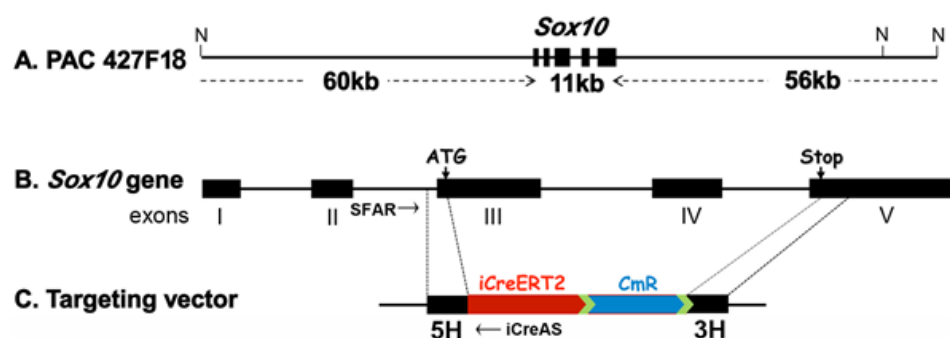


Figure 1 (A) PAC clone RP21-427F18 spans the Sox10 gene with upstream and downstream flanking regions; (B) Diagram of the Sox10 gene locus; (C) The targeting vector used for homologous recombination. N: NotI site; CmR: chloramphenicol resistance cassette; green chevrons: frt sites; 5H: 5' homology region; 3H: 3' homology region. The positions of genotyping primers (SFAR and iCreAS) are indicated.

We modified a mouse phage artificial chromosome (PAC) clone (RP21-427F18, UK Human Genome Mapping Project Resource Centre; Fig. 1) that contains 127 kb of genomic DNA spanning the Sox10 gene locus. The targeting construct replaced the Sox10 ORF with a cassette encoding a tamoxifen-inducible form of codon-improved Cre recombinase (iCreER^{T2}) (1,2). The 5' and 3' homology regions were first amplified by PCR using the PAC clone as template, then sub-cloned into the targeting vector. A chloramphenicol resistance (CmR) cassette flanked by frt sites was inserted between iCreERT2 and the 3' homology sequence. PAC targeting and the subsequent removal of CmR were performed in E.coli strain EL250 by standard methods (2). The modified PAC, with the Sox10 open reading frame replaced by iCreERT2, was linearized with NotI and the desired 120 kb band purified by pulsed-field gel electrophoresis for pronuclear injection. Genotyping was by PCR with forward primer specific to intron 2 of Sox10 (SFAR:5'-TTGCGATGGGAGAGTCTGAC) and reverse primer specific to iCreERT2 (iCreAS:5'-AGGTACAGGAGGTAGTCCCTC) producing a 742 bp product. Four independent Sox10-iCreERT2 founders were obtained and one selected for further use on the basis of specificity and efficiency of recombination of the Rosa-YFP reporter. Approximately 65% of Sox10-immunoreactive cells in the cerebral cortex and corpus callosum, and >90% in the spinal cord, were YFP+ one week after tamoxifen administration to Sox10-iCreERT2 : Rosa26-YFP animals (not shown).

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References

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