S100a4-CreERT2-PolyA TG Strategy

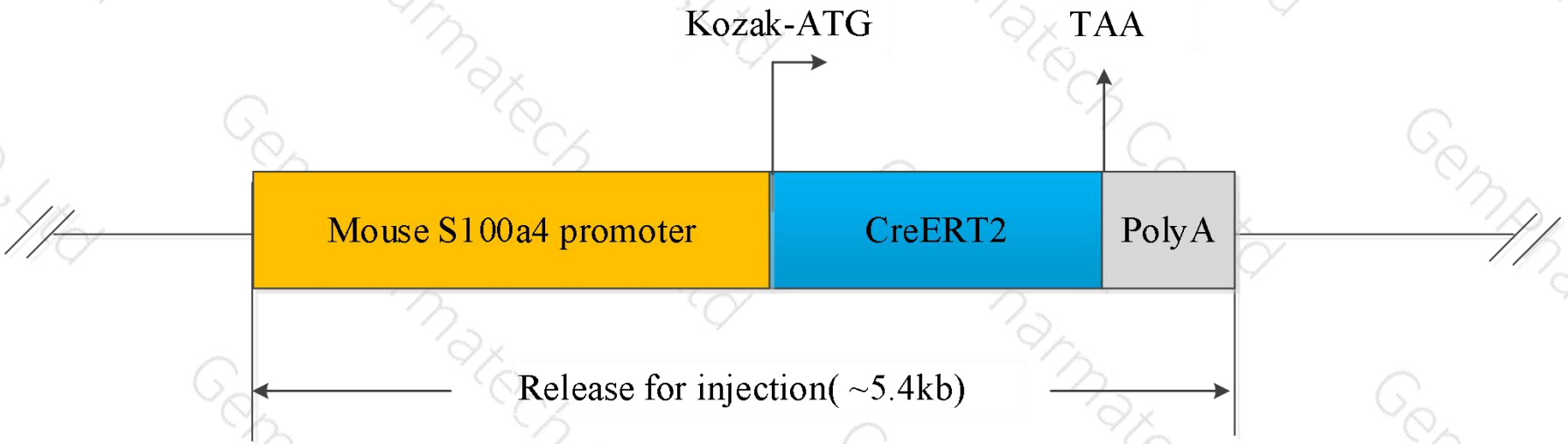
Designer:	**Xiaojing Li**
Reviewer	**Jia Yu**
Design Date:	**2019-11-14**

Project Overview

Project Name	***S100a4-CreERT2-PolyA***
Project type	**TG**
Strain background	**C57BL/6J**

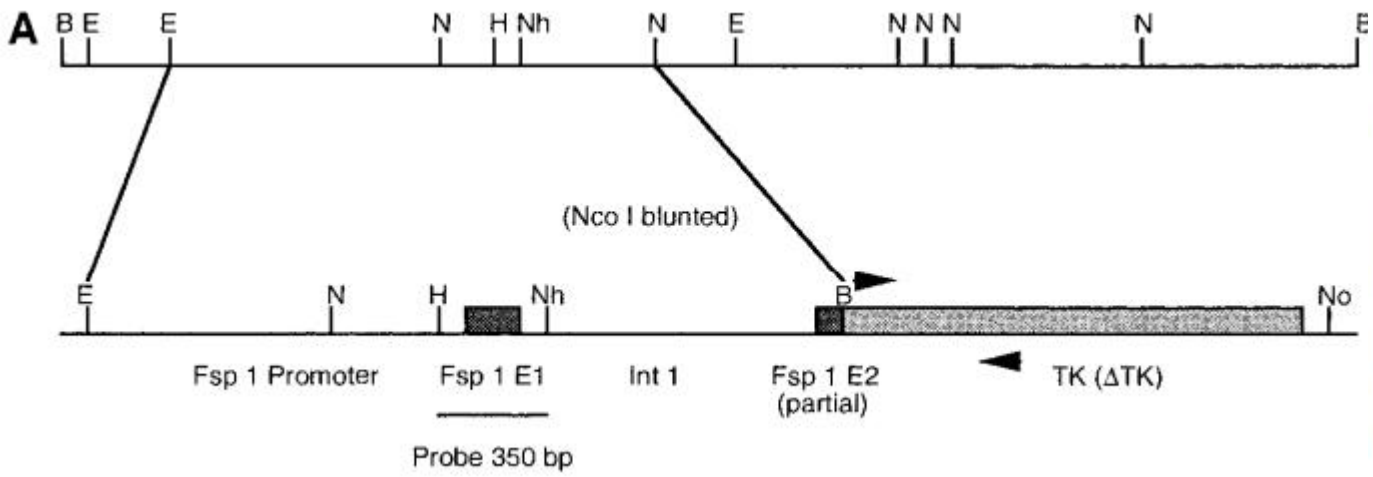
TG strategy

This mice model is made by transgenic technology, and the schematic diagram is as follows:



- Kozak：Kozak sequence (GCCGCCACC) will be added before the ATG，in order for the ribosome to recognize the initiation codon.

Promoter Sequence of Mouse *S100a4(Fsp1)*



Material and Methods

Generation of an FSP1.ΔTK minigene for transfections and production of transgenic mice. A portion of the FSP1 genome which contains 2.5 kb of the FSP1 5′ flanking region as well as the first intron and the noncoding portions of the first and second exon (see Fig. 1A) extending from the *Eco*RI site (−2.5 kb) through to the *Nco*I site (+1.2 kb) was ligated into the polylinker of pBSII plasmid such that *Bam*HI sites were present at both 5′ and 3′ ends (26). During this process the *Nco*I site was destroyed by mung bean enzyme digestion. The thymidine kinase gene fragment was added next by ligating this *Bam*HI–*Bam*HI fragment into the *Bam*HI and *Bgl*II sites of

FIG. 1. Gancyclovir sensitivity of fibroblasts and epithelium containing FSP1.TK(ΔTK) minigenes in culture. (A) FSP1.TK minigene construction. FSP1.TK(ΔTK) constructs were assembled as described in the gene map; B, *Bam*HI; N, *Nco*I; E, *Eco*RI; No, *Not*I; H, *Hin*dIII; Nh, *Nhe*I; arrows, PCR primer regions used to truncate the TK coding sequence to ΔTK. (B) 3T3 fibroblasts and MCT tubular epithelium stably transfected with FSP1.TK or control plasmids. Each cell line was cultured

[1]Iwano M; Fischer A; Okada H; Plieth D; Xue C; Danoff TM; Neilson EG. 2001. Conditional abatement of tissue fibrosis using nucleoside analogs to selectively corrupt DNA replication in transgenic fibroblasts. Mol Ther 3(2):149-59.

Mouse *S100a4(Fsp1)* Promoter Sequence(3438bp)[1]

GAATTCCCAGGTGTTGTGATGGGAAACGATGGGAGGGAGTCAGAGTCAGAGGGAGGCTCCCAGTTGGGGTGAAGGTGGTAGATATTCTGCTCCTGAGGAATTTATGGTGATGTCAG
GGAACTGAAACTTCTGTCTAGAAAAATCACAACAGGCACAGCGGGGAGGTGAATTGATTCCTTGCGAGTCATACCCTTGTTTTGTGACAGTGAGGATGACCCGGCCCCTGGAGCAG
GCAGTAGCTGCCATCGTGTGCACCTTCCAGGAGTATGCAGGGCGCTGTGGGGATAAATACAAGATCTGCCAGTCGGAGCTCAAGGAGTTGTTGCAGAAGGAGCTGCCCACCTGGAC
GCCGGTGAGCACCTCATATCTCTCCCCACTTGGACTCTGCAAATCGTGGCCTAGGGCCAAGCAGCAGCATAGGGTAGCACATGAGCTGAGACACAGGGCTGGGAAGGGGGACTGA
AATGGGTGTCTTATCAGGTGGGTAGGATGAGGTTGGCCCCATGAGGGTTTTGGTTTGGAATGGGGCAGCCTAACCATCGGAGGGGAGTGGTGTGGGAAGCTACTTTGGCTCCTTATT
CTGGTTTCCTGACCCTGCCTTCTCATCCCTTCCACAGAGTGAGTTCCGGGAGTGTGACTACAATAAATTCATGAGTGTTCTGGATACCAACAAAGACTGCGAAGTGGACTTTGGGGA
GTACGTGCGCTCACTTGCCAGCCTCTGTCTCTACTGCCACGAGTACTTCAAAGAGTGCCCCCCTGAGCCTCCTTGCCCCCAGTAGCCTCTGATCCAAAGGTGTACGCTATCCCAGAAG
GGCAGGGTCTGCTCCAGTCCTCCATCTTTGTCCTTGAGGTGGTCCTGGGTGTGTAGCCACACCCTTCCCACTCTCTCTGTGGTACCCCTTTCAATCTAGACTTGCCAAGTTCTTGATGT
GCTAACCCCACCCAGCTACCCATGAGCTTTCGAGGCTTTCCTAGGGATGTCTAGCTTGTGAGGGGTGGGACAGTAGCCAGCCTTTGCCGCTTCTCTTCTTGGAAGGGAAGAACATCT
CTGCTCAGCCATGTGCACACAACTGGGACCGCTGTGGCAGGGGCCTCCTCAACTCCCAATAAAGAAATGTCTTCTTGGCTTACTTTTGTTTTTTTCTGATGGGACACACTGGGCCTTG
GGACCGAGTCCTTGTTCCTTTATGCTCCTTACTACTGGAGGTAGGAGGCTTACCATGGAAGGCATGGACCCCCAAAGCGGTGTCAGGCCCTGTAGAAATGCACACATTTCAGGAGGG
TAGGGGTAACACGTGTCCTATCAGATGAGACTGGAGGGTCTCTGTCTCTCTCTGTCCCCTGTCTTGAGATAGAAGTCCTTATCTTGGACTTTCAAGGAGGACAAGGGGCTCCTTGGG
AGGTACTTCTGACCAGATGCTGCAAGGAGAGTATGGTTGTGGGAGCCCAAAGCCAAACCTCCATCTAACCTTCACTCAATCCCCGAATTTGTACCCTATCCTTAGAGATTAATCCTG
ACTCCCCCTTTTACCTATTTCCTCTTTAACTCTCTTCTTCAAGCTGAACATTCAACCCCGAATGCTCCTGTCATTCCTCAATATCCTTACTCCAGCTTCCATCCATTCGAAAACCTCCAG
GCCACACTGCCACCCTAACTCCATCATGGCCTCCTAGGTATAGCTCCTACTTCATACCTGGGGTGGTCCCAAGGTCCCTCCTGACTTGCTAGCCTCTATCCTGGGTCTTCCTGATTGTG
ACAAGAAGCTGTTTAGGCTGGAGGGAAGTGCTGACATTGTCCCACTGGCTGGGGTCACCTCCTTCGTTCCTGGGCCACATATTTCCAGGGCAGCTCCTTATCCCTTGCCCATAACATC
TCCATCTCCTTTCCTGTGGCCCACACCTCATGTCCAGGTTGCCCGTTCTCAAAGCTTCCTAAACTTCTGGCTGAGCTGTGGCTGCTTGGTGGTGTCCACCCCATCCAAGTCTCTGCCGT
GCCCACTGGAGCTCACTCACTACTTGATTGTGCCTGCTGGGGAGGGAGCAGGAAGCCTGGTTCCCAGACTGGGCTGGTCGAGGGTGCTATGACATTTACTACATCAACCAACAGCA
AGAGCACAGTATCCATGTTCCCCCATCCTCTGCATGGGCAGGGCCTGGCAGGGTATAAATAGGTCAGATTGTTGGGCTCTCCCCAAACCTCTCTATTCAGCACTTCCTCTCTCTTGGT
CTGGTGAGTTGTGTTGGTCTGATAGCACTGCTAGCGGCATTAGAGGCTGAGGCTAGGGTAGAAGAAAGGGGGGCTGCTGGGGGAACAGATGTCTTTAATAAATCCAGATGAGAGAT
TCTGATGTGGAGGTTCATGTATGTGTGTGTGTGTGTGTTTTTCACGAGAATGAAAACCAAAAAAAAAAAAAAAAAAAAAAAGTGTATAAATGGCTACATCTGAGCTCCCGGA
GGTTTTGAGATACTGAGGCTGGCTTGCATGTTGCTATAGTGTATATTGGTGGTGCTTGGGAGTCACTGTCATGCATAGGATGCTGACTCGTGTTGCTGGGTAATACAAGACAGTGTGT
GGACACTCGGGTACAGGAAGCAAAGCGAAGGCATCAGTAGGCCTTTTTGTTTTACAGTATTTAAATTACAGTTTTTATTTGTGTGTATGAGCGTATGGGTTGGGCTGGAGCAAATGC
CAAGGCGACATTGTGGGAGCCAAAGGACAATTTGTGTGGGAGTCAATCTGTTCCTTCTAGCATGTGGGCTGTGGGGATCAAACTCAGGCCCTGGAGCTTGGTGGCAAGCACCTCTA
CCCACTGAGCTATCTCTCCAGCACCCTCCTGCAGGCATTCGTGTTTGTAGTGTGTCTTATTTTTAATAGCCCTATGAACATATAGCACCTAGGCCAAGAAAGCCTGGCTTCCCCACCC
TCTCCTCTTGCATCCCTACCTCTGCCACTTCATCTTACTCCTATTAGGCAGCTGGGGTTTTTCCACTTTTTTTTGTCTGCCTCTGGGCAGGCAGCCAGCAGCCGCGCCCAACGCTGGGA
GGGAGAAGAATGGGCCAGGGCCTGGTGCTTGTGGTTGAGCTGTGGGAGTGAGTAAGCTGATGGAAAACTGCTGTTGTTGAGGCCAGGACTGAGAGGCACAGAAAGGTGCTGGCAT
GGATCTCCAGAGTTTGAGGGGTAGGCTTTGCAGGTTTCAGAGCCCAGAGCACATGTGACCTTCTTGCCATCAATGGGTCCCATTCCTCTGATCTCCCCAGGGGGTGAGGTCCATCTCT
TAGAGAGTTGGCTGGGATAGAGCACTTAAAATGGGGACAGAATGAGTGTGATTTGGGTCATGCTCAGCAACACATATCCAGTTCTCAACACACTGTTGGCGTGGGTTGGAGAATGT
TACTTTTGTGTCTCCTGCCCTTAGGTCTCAACGGTTACC

Technical routes

- The *S100a4* promoter[1] is from references, the length is about 3.5kb.

- In this study, the transgenic vector was constructed in vitro, and transgenic fragments containing *S100a4-CreERT2-PolyA* were micro-injected into the fertilized eggs of C57BL/6J mice, and pcr-positive F0 generation (i.e., founder) mice were obtained.

Notice

- Transgenic fragments injected into the prokaryotes will be randomly integrated into the mouse genome. Affected by the insertion site and copy number of transgenic fragments, the expression level of transgenic mice may be different.

- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

如您有任何疑问，欢迎垂询。

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd