Acta2(αSMA)-iCre-PolyA TG Strategy

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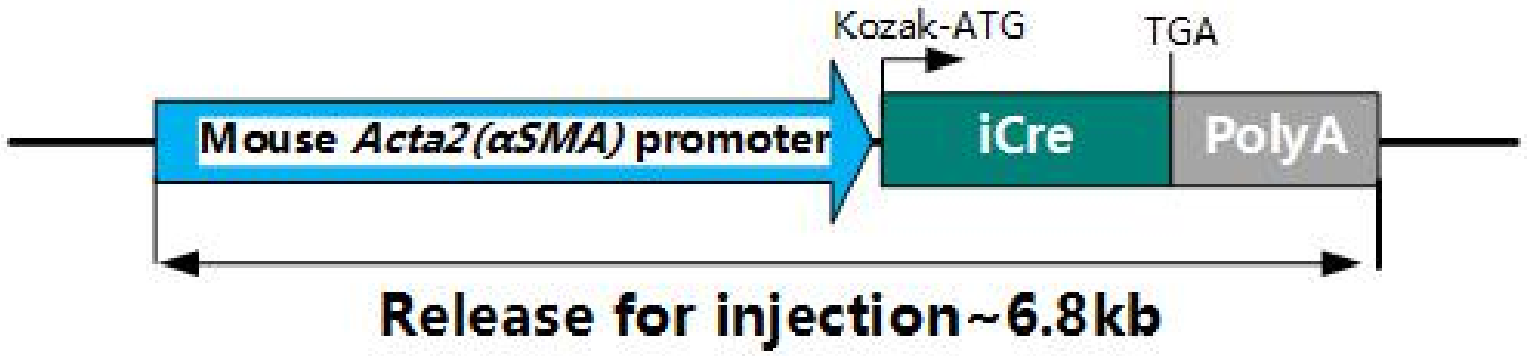
Project Overview

Project Name	***Acta2-iCre-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) [1] will be added before the ATG , in order for the ribosome to recognize the initiation codon.

Summary of Mouse *Acta2(αSMA)* Promoter[1][2]

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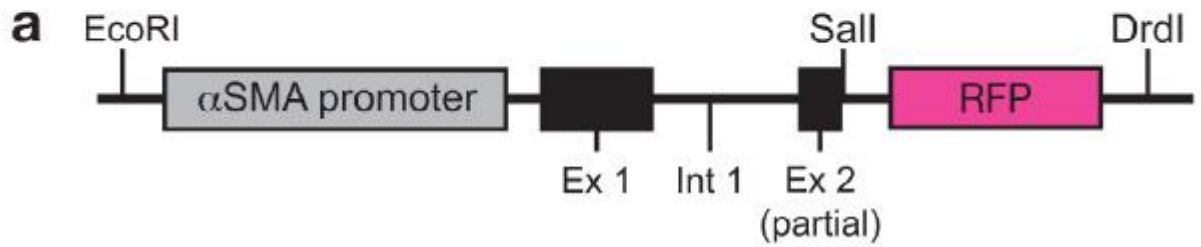
Supplementary Figure 1



Generation of αSMA-RFP transgenic mice

αSMA-RFP mice were generated using an extended *αSMA* promoter containing approximately 2.4kb of the *αSMA* promoter plus exon 1, intron 1, and part of exon 2 (altogether 5.2kb) amplified with the following primers: 5' CAATGCATGCTGTACAAACATCAGG 3' (Forward); 5' AGCTGGAGCAGCGTCTCAGGGTTCTGC 3' (Reverse). The extended *αSMA* promoter was cloned into the pDsRed-Express-1 vector (Clontech) using EcoRI and SalI restriction sites, and the whole αSMA-RFP construct was released from the vector using EcoRI and DrdI before purification and injection into fertilized eggs. All transgenic mice were created in the Brigham and Women's Hospital

1. Lebleu VS; Taduri G; O'Connell J; Teng Y; Cooke VG; Woda C; Sugimoto H; Kalluri R. Origin and function of myofibroblasts in kidney fibrosis. *Nat Med.* 2013 19 (8) 1047-53
2. Valerie S LeBleu, Yingqi Teng, Joyce T O'Connell, David Charytan, Gerhard A Müller, Claudia A Müller, Hikaru Sugimoto & Raghu Kalluri.Identification of human epididymis protein-4 as a fibroblast-derived mediator of fibrosis. *Nat Med*. Feb;19(2):227-31. doi: 10.1038/nm.2989. Epub 2013 Jan 27.

Summary of Mouse *Acta2(αSMA)* Promoter[1][2]

CAATGCATGCTGTACAAACATCAGGACTGGAACTCTGGTCCCCCAAATCCACATAAAAACCTGGCAGATATGCGTTGGCTGGAGCTGACTGGCTAGCTAGGTGAGCCAAATCAGTAAGCACTGGGTTCAAGTGAGAGATCCTGTCTAAT
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TGAACTGCAACCAAATGACCAGATGTGCTCCCTCTAATCAGTCCATAACTCCTTGCATTCATGGCTGCCAACTCTGGGAGTTATCTAAGCACTGGATGGAACTCTGCTTGTATATTTGTACACACTTATCCAATGGGGCTTTCTCTCACGG
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CTAAAATAACACAACAGGACCAAGAACCCTGTCTGTGGGTGGGAGTGACTAGGCTCTAGCCAAATGCTCTGCGCTACAGTAGCTTCTCGCTCGCTGTCTCTGCAGAACCCTGAGACGCTGCTCCAGCT

We choose 5229bp fragment of Mouse *Acta2* Promoter to drive *iCre* expression according to the reference described.

Technical routes

- The *Acta2* promoter[1][2] is from references, the length is about 5.2kb.

- In this study, the transgenic vector was constructed in vitro, and transgenic fragments containing *Acta2-iCre-polyA* were micro-injected into the fertilized eggs of C57BL/6J mice, and pcr-positive F0 generation (i.e., founder) mice were obtained.

Notice

- According to the existing JAX data,Cre activity is detected in a few interstitial renal cells of healthy mice. In a model of experimentally induced renal fibrosis (by unilateral ureteral obstruction), Acta2 expressing fibroblasts that accumulate in fibrotic kidney interstitium exhibit Cre activity.

- 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.

Targeted Progress (from Jax)

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Detailed Description

These aSMA-Cre transgenic mice express Cre recombinase under control of an extended 5.2kb mouse *Acta2* (actin, alpha 2, smooth muscle, aorta) gene promoter that includes the *Acta2* promoter plus exon 1, intron 1, and part of exon 2. Cre activity is detected in a few interstitial renal cells of healthy mice. In a model of experimentally induced renal fibrosis (by unilateral ureteral obstruction), *Acta2* expressing fibroblasts that accumulate in fibrotic kidney interstitium exhibit Cre activity. The donating investigator reports Cre activity is detected in *Acta2* expressing myofibroblasts with minimal Cre activity in striated muscle. Hemizygotes are viable and fertile. Homozygous viability/fertility has not been tested (October 2017).

In an attempt to offer alleles on well-characterized or multiple genetic backgrounds, alleles are frequently moved to a genetic background different from that on which an allele was first characterized. It should be noted that the phenotype could vary from that originally described. We will modify the strain description if necessary as published results become available.

https://www.jax.org/strain/029925

Allele Name	transgene insertion 1, Raghu Kalluri
Allele Type	Transgenic (Recombinase-expressing)
Allele Synonym(s)	aSMA-Cre
Gene Symbol and Name	Tg(Acta2-cre)1Rkl MGI , transgene insertion 1, Raghu Kalluri
Gene Synonym(s)	
Promoter	*Acta2*, actin, alpha 2, smooth muscle, aorta, mouse, laboratory
Expressed Gene	*cre*, cre recombinase, bacteriophage P1
Site of Expression	Cre activity is detected in *Acta2* expressing myofibroblasts with minimal Cre activity in striated muscle.
Strain of Origin	FVB
Chromosome	UN
Molecular Note	The aSMA-cre transgenic construct containing the Cre recombinase gene under control of an extended 5.2kb mouse Acta2 (actin, alpha 2, smooth muscle, aorta) gene promoter that includes the Acta2 promoter plus exon 1, intron 1, and part of exon 2, was microinjected into FVB fertilized mouse eggs. The founder line 1 was subsequently established.

如您有任何疑问，欢迎垂询。

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