Tagln-iCre-polyA Cas9-KI Strategy

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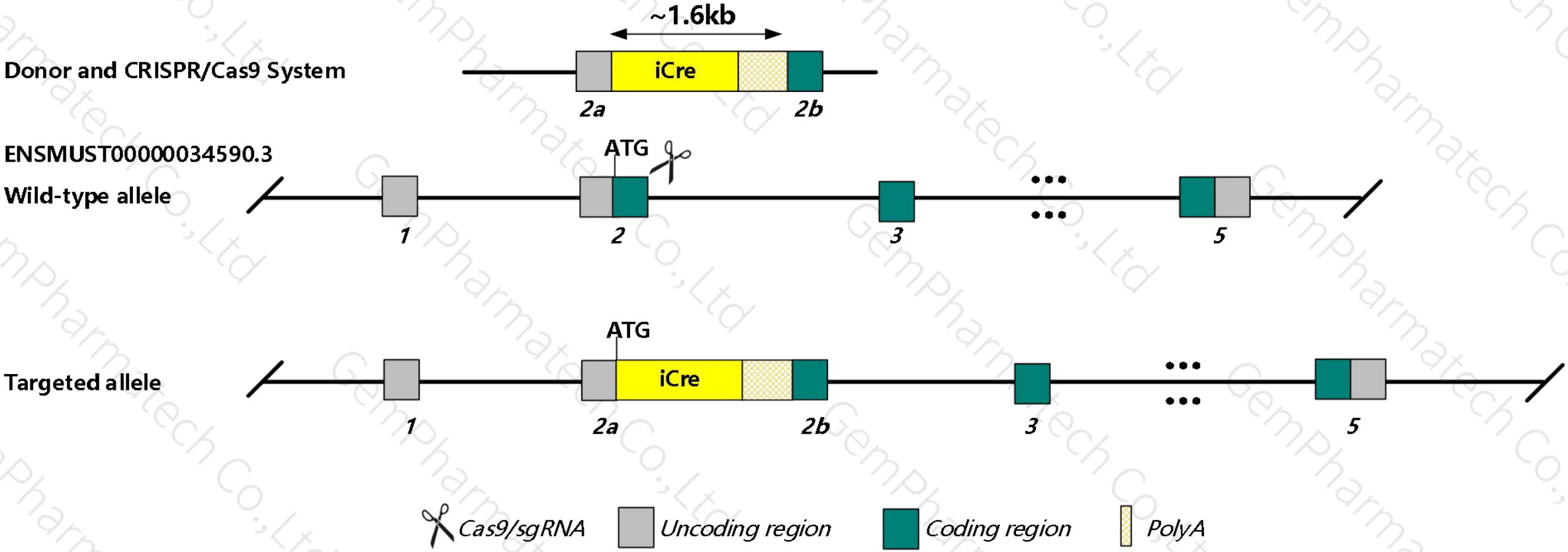
Reviewer	**Xiaojing Li**

Project Overview

Project Name	***Tagln-iCre-polyA***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Tagln* gene. The schematic diagram is as follows:



Technical routes

- The *Tagln* gene has 3 transcript.According to the structure of *Tagln* gene, *Tagln-201*(ENSMUST00000034590.3) is selected for presentation of the recommended strategy.

- *Tagln-201* gene has 5 exons, with the ATG start codon in exon2 and TAG stop codon in exon5.

- We make *Tagln-iCre-PolyA* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near start coding(ATG) of Tagln gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in iCre-PolyA after start coding(ATG) of Tagln gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, Mice homozygous for targeted mutations in this gene are viable, fertile and phenotypically normal.
- Insertion of iCre may affect the regulation of the 5' end of the *Tagln* gene.
- According to the existing JAX data, Cre recombinase activity is shown in adult smooth muscle cells (such as arteries, veins, and visceral organs) and cardiac myocytes, but activity is not observed in the same embryonic tissues.
- There will be 2 to 4 base mutations in exon2 of *Tagln* gene in this strategy.
- The *Tagln* gene is located on the Chr9. If the knockin mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

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Tagln transgelin [*Mus musculus* (house mouse)]

Gene ID: 21345, updated on 13-Aug-2019

Summary

Official Symbol	Tagln provided by MGI
Official Full Name	transgelin provided by MGI
Primary source	MGI:MGI:106012
See related	Ensembl:ENSMUSG00000032085
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Sm22; Sm22a; Ws310
Summary	This gene encodes a smooth muscle cell-specific cytoskeletal protein. The encoded protein is structurally similar to calponin, an actin-binding protein. In mouse models of atherosclerosis the gene product may be involved in plaque cell and atherosclerotic lesion formation during atherogenesis. [provided by RefSeq, Mar 2010]
Expression	Biased expression in bladder adult (RPKM 3112.1), stomach adult (RPKM 1002.7) and 5 other tissues See more
Orthologs	human all

Genomic context

Location: 9 A5.2; 9 25.36 cM

See Tagln in Genome Data Viewer

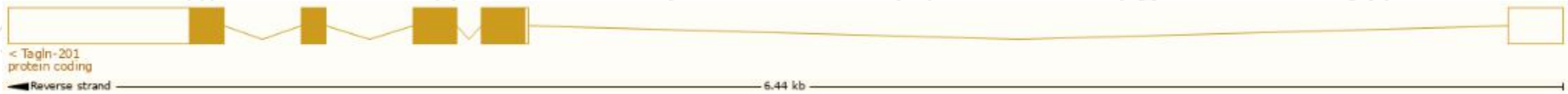
Exon count: 5

Transcript information （Ensembl）

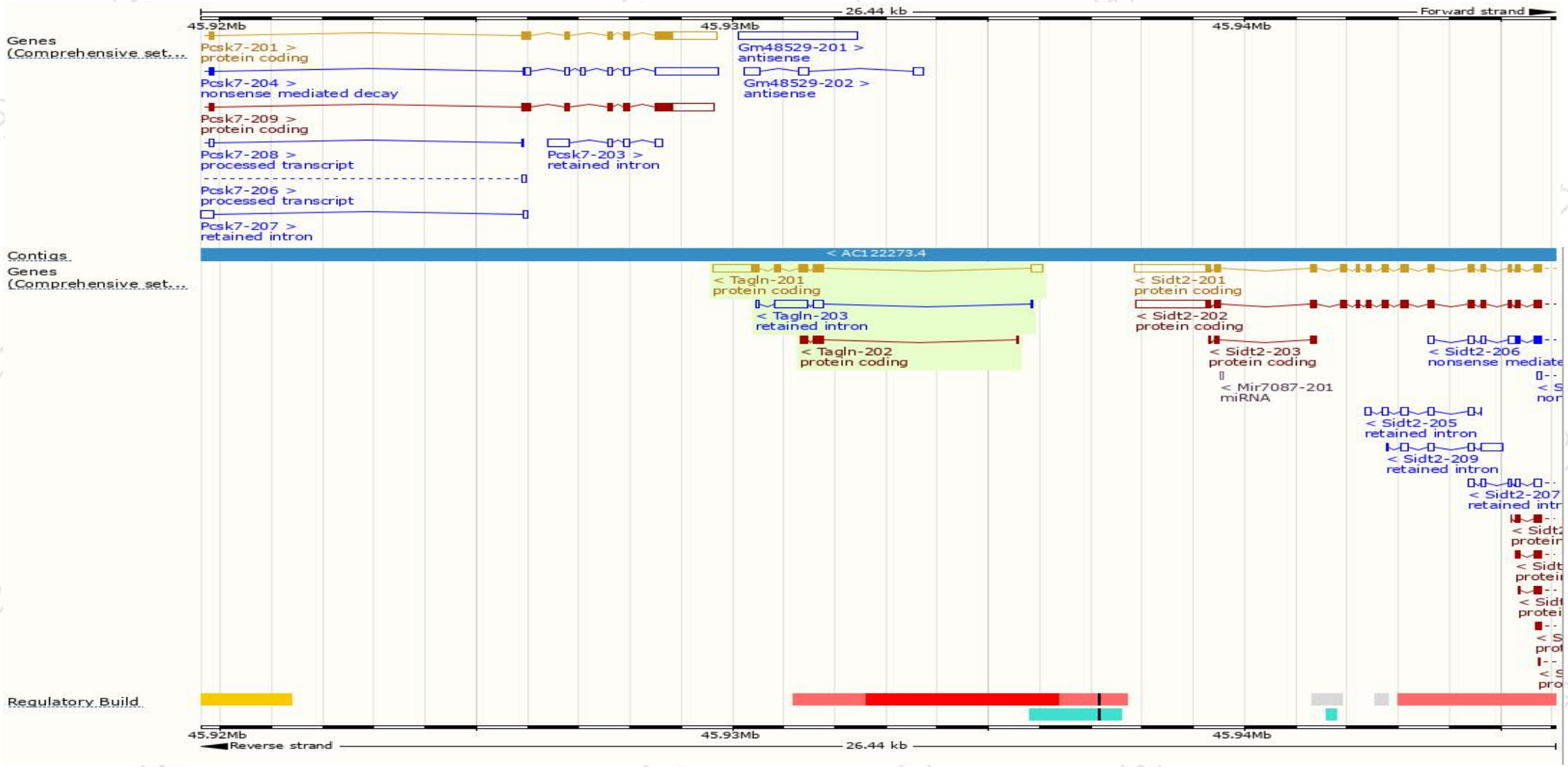
The gene has 3 transcripts, and the transcript is shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tagln-201	ENSMUST00000034590.3	1595	201aa	Protein coding	CCDS23137	P37804	TSL:1 GENCODE basic APPRIS P1
Tagln-202	ENSMUST00000215509.1	369	109aa	Protein coding	-	A0A1L1STN8	CDS 3' incomplete TSL:3
Tagln-203	ENSMUST00000216035.1	933	No protein	Retained intron	-	-	TSL:5

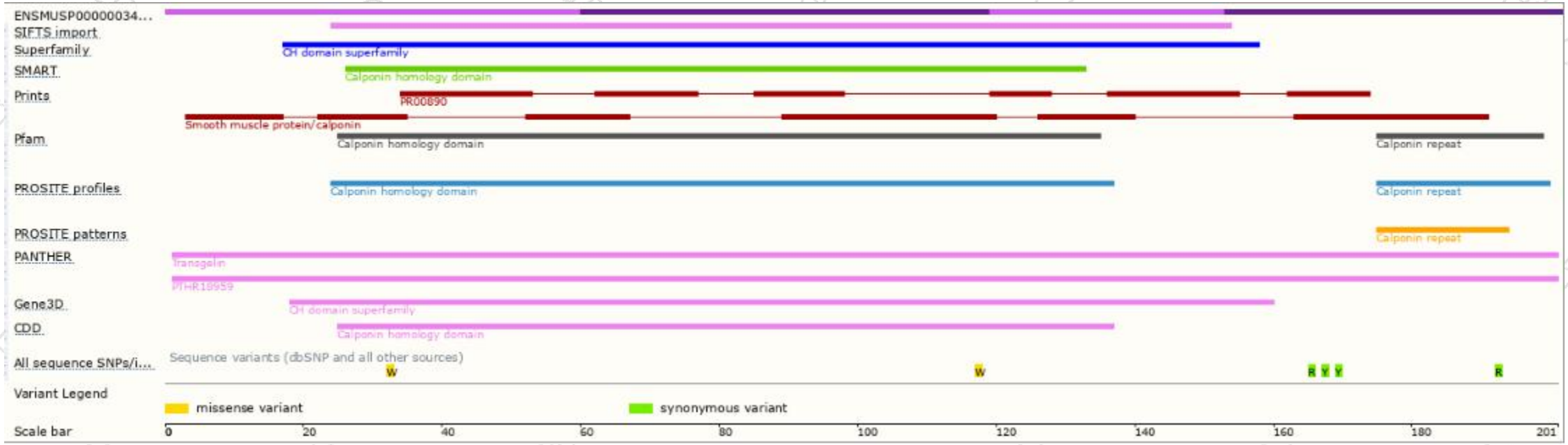
The strategy is based on the design of *Tagln-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



Mouse phenotype description(MGI)

Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/marker/MGI:106012) .

Mice homozygous for targeted mutations in this gene are viable, fertile and phenotypically normal.

Targeted Progress (from JAX)

The Jackson Laboratory

B6.129S6-Tagln $^{tm2(cre)Yec}$/J MOUSE STRAIN DATASHEET - 006878

Detailed Description

Mice homozygous for this SM22alpha-CreKI allele are viable and fertile. These mice have a Cre-recombinase gene inserted into the endogenous transgelin (SM22alpha) locus. The donating investigator reports that this mutation results in a loss of function of the targeted gene. Cre recombinase activity is shown in adult smooth muscle cells (such as arteries, veins, and visceral organs) and cardiac myocytes, but activity is not observed in the same embryonic tissues. It has been the experience of The Jackson Laboratory that optimal breeding is achieved by mating heterozygous females to homozygous males as female mortality post gestation has been noted in our colony. These SM22alpha-CreKI mice may be useful for Cre-lox technology applications in studying smooth muscle and cardiac gene function, as well as cardiovascular disease.

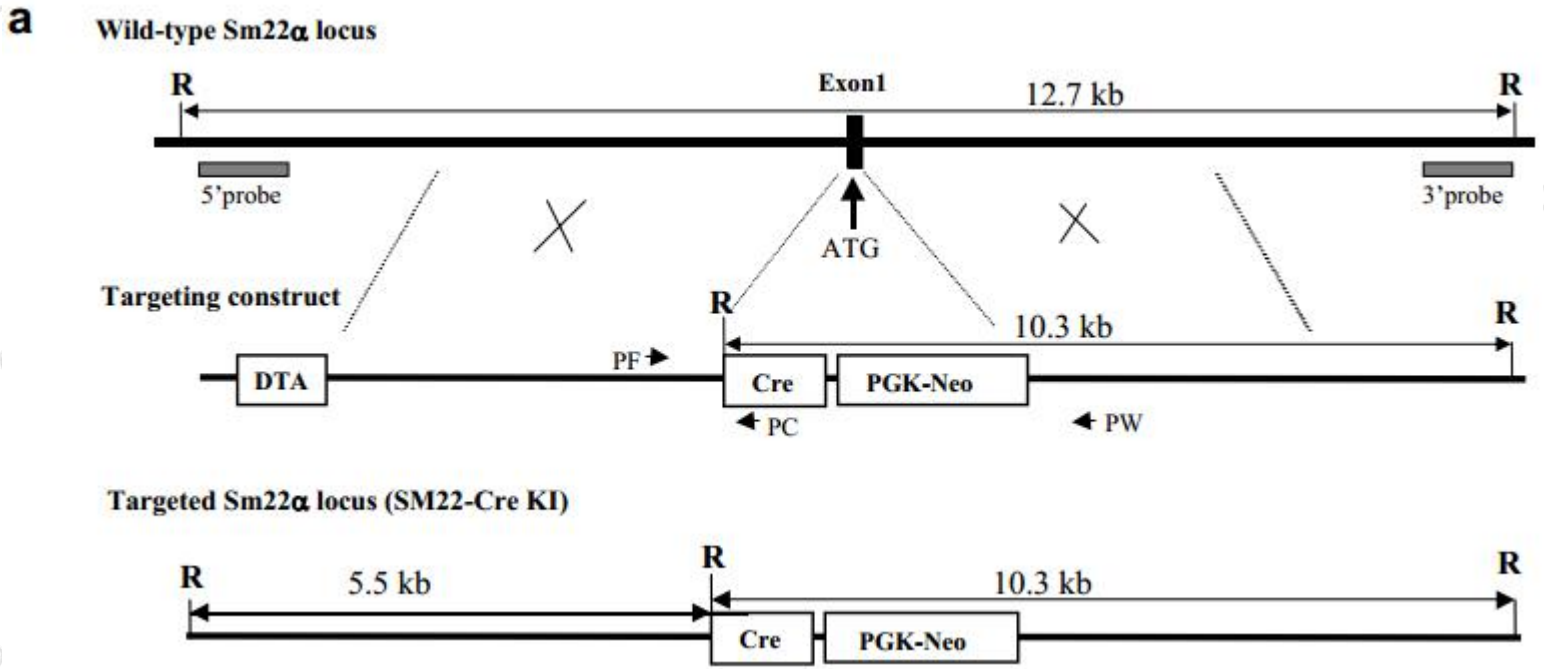
Expression Data

Expressed Gene	cre, cre recombinase, bacteriophage P1
Site of Expression	adult smooth muscle cells (such as arteries, veins, and visceral organs) and cardiac myocytes

Zhang J; Zhong W; Cui T; Yang M; Hu X; Xu K; Xie C; Xue C; Gibbons GH; Liu C; Li L; Chen YE. 2006. Generation of an adult smooth muscle cell-targeted Cre recombinase mouse model. Arterioscler Thromb Vasc Biol 26(3):e23-4.

https://www.jax.org/strain/006878

References



This SM22α-CreKI mouse line was generated by knocking in the Cre-recombinase coding sequence into the endogenous SM22α gene locus via homologous recombination of embryonic stem cells (supplemental Figure S1, available online at http://atvb.ahajournals.org). Consistent with previous reports of SM22α knockout mice, our SM22α-CreKI heterozygous and homozygous mice were fertile and appeared phenotypically normal.

If you have any questions, you are welcome to inquire.

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