Scx Cas9-KO Strategy

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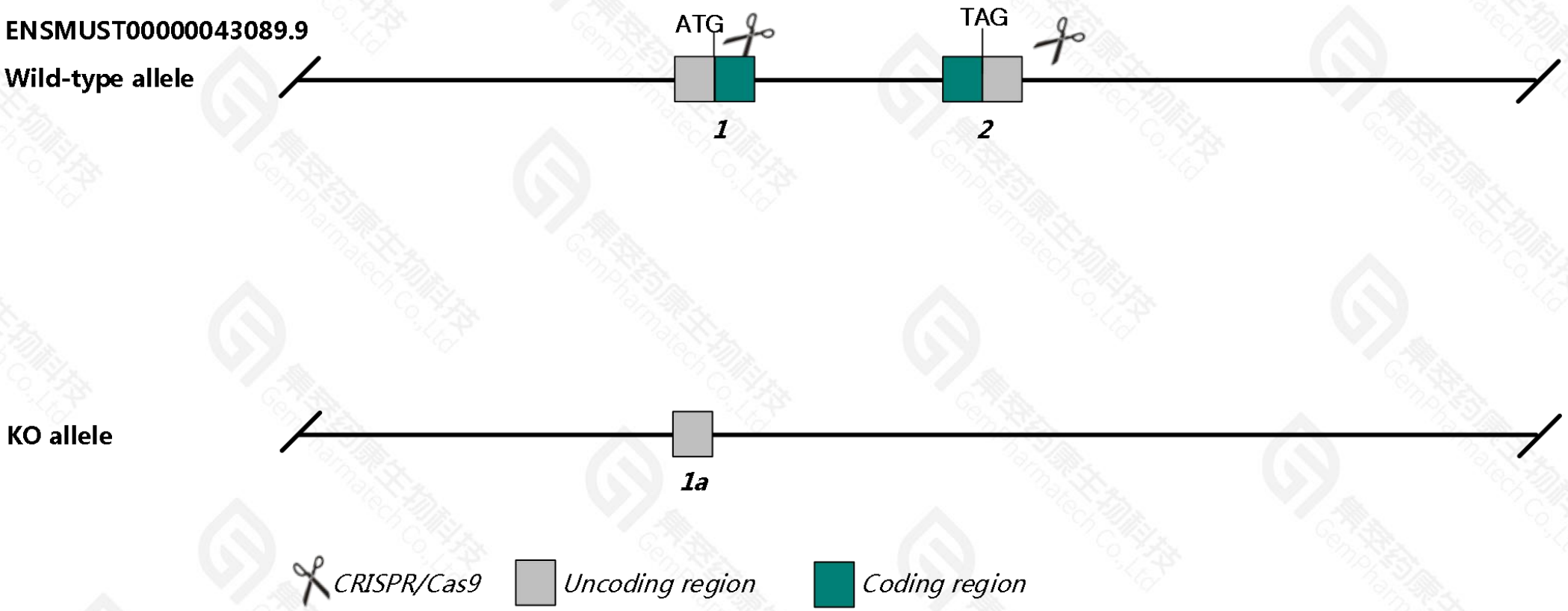
Design Date: 2021-9-24

Project Overview

Project Name	***Scx***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

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



Technical routes

➢ The *Scx* gene has 2 transcripts. According to the structure of *Scx* gene, exon1-exon2 of *Scx-201*(ENSMUST00000043089.9) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Scx* gene. The brief process is as follows: CRISPR/Cas9 system were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

Notice

➢ According to the existing MGI data,homozygotes for a targeted mutation develop normally up to E6.0-E6.5, but become arrested and fail to gastrulate and form mesodermal cells. In chimeric embryos, mutant cells are excluded from sclerotome-derived chondrogenic lineages but contribute to other cell types, including mesodermal tissues.

➢The flox region is in the intron of the *Bop1* gene, which may affect the regulation of this gene.

➢ The *Scx* gene is located on the Chr15. If the knockout 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 biological processes,all risk of the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Scx scleraxis [Mus musculus (house mouse)]

Gene ID: 20289, updated on 13-Mar-2020

Summary

Official Symbol	Scx provided by MGI
Official Full Name	scleraxis provided by MGI
Primary source	MGI:MGI:102934
See related	Ensembl:ENSMUSG00000034161
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	BB114693, Bhlha41, Scl
Summary	This gene encodes a basic helix-loop-helix type transcription factor involved in mesoderm and heart valve formation. The encoded protein is expressed during embryonic development of tendons and ligaments. The gene product regulates collagen type I gene expression in cardiac fibroblasts and myofibroblasts, and it may play a role in myocardial remodeling. The protein is expressed in the scar area of the adult heart following myocardial infarction. [provided by RefSeq, Feb 2010]
Expression	Biased expression in adrenal adult (RPKM 33.4), limb E14.5 (RPKM 25.3) and 13 other tissuesSee more
Orthologs	human all

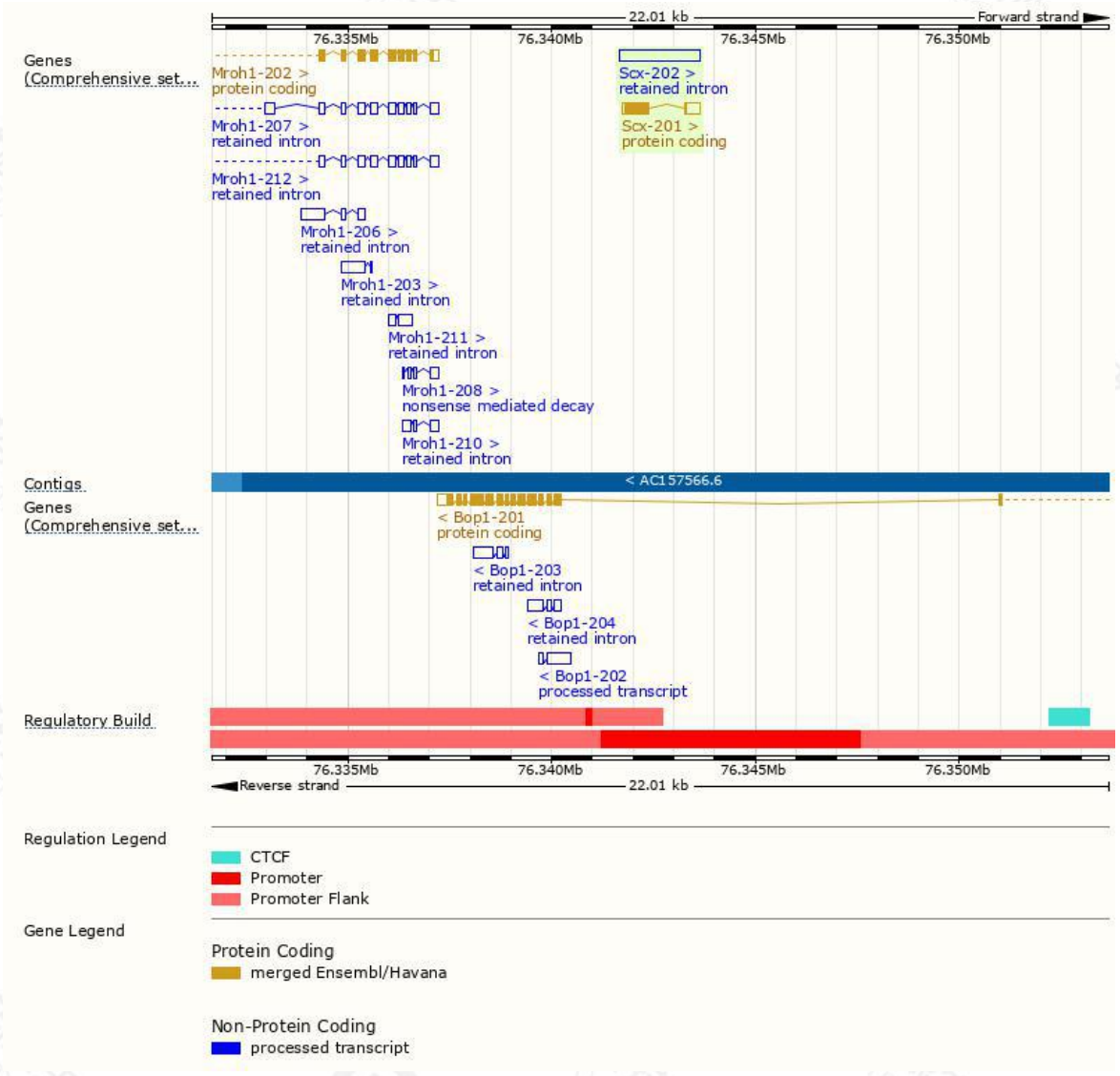
Transcript information（Ensembl）

The gene has 2 transcripts,all transcripts are shown below:

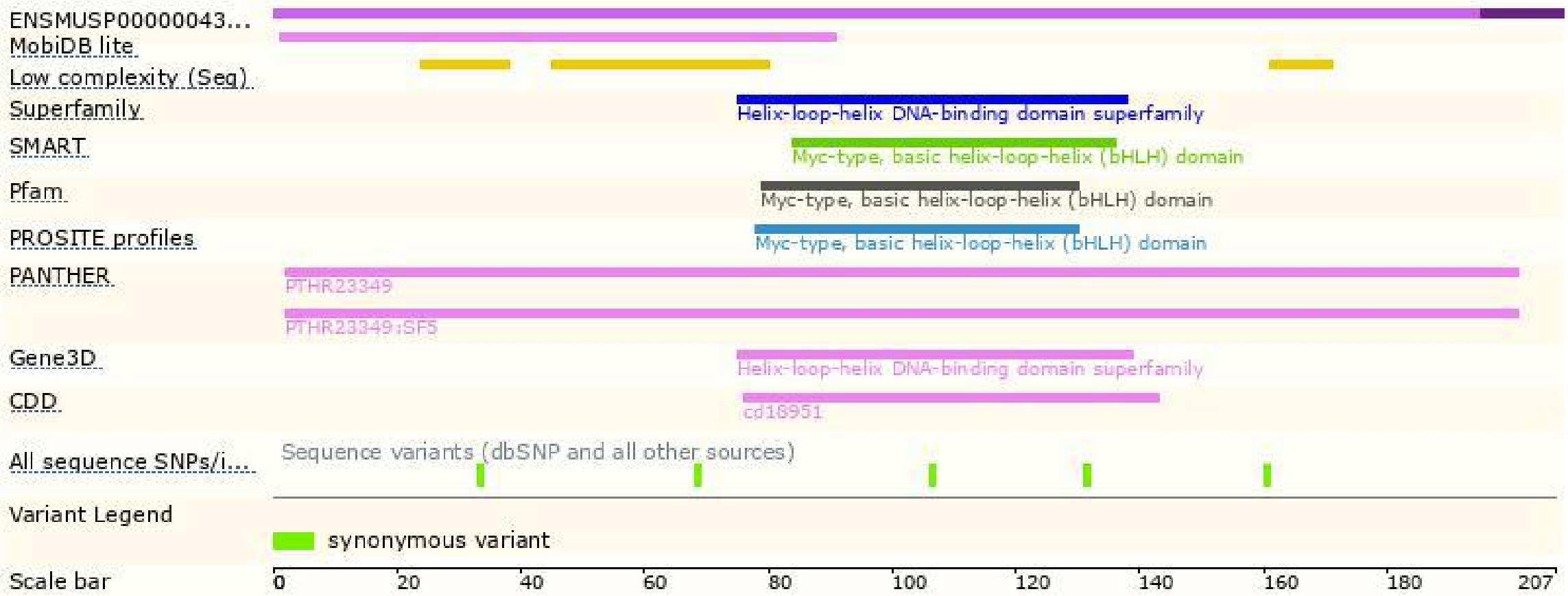
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Scx-201	ENSMUST00000043089.8	1035	207aa	Protein coding	CCDS27571	Q53ZC3 Q64124	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Scx-202	ENSMUST00000229271.1	2007	No protein	Retained intron	-	-	

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

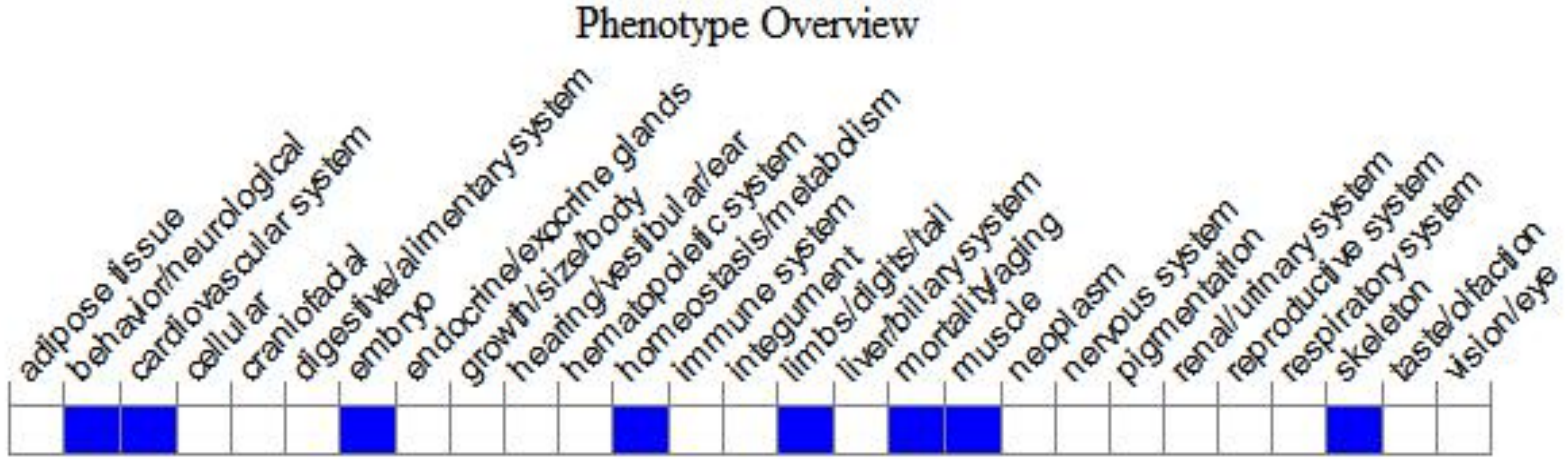
Genomic location distribution



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/).

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