Fsip2 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Daohua Xu

Design Date: 2020-7-15

Project Overview

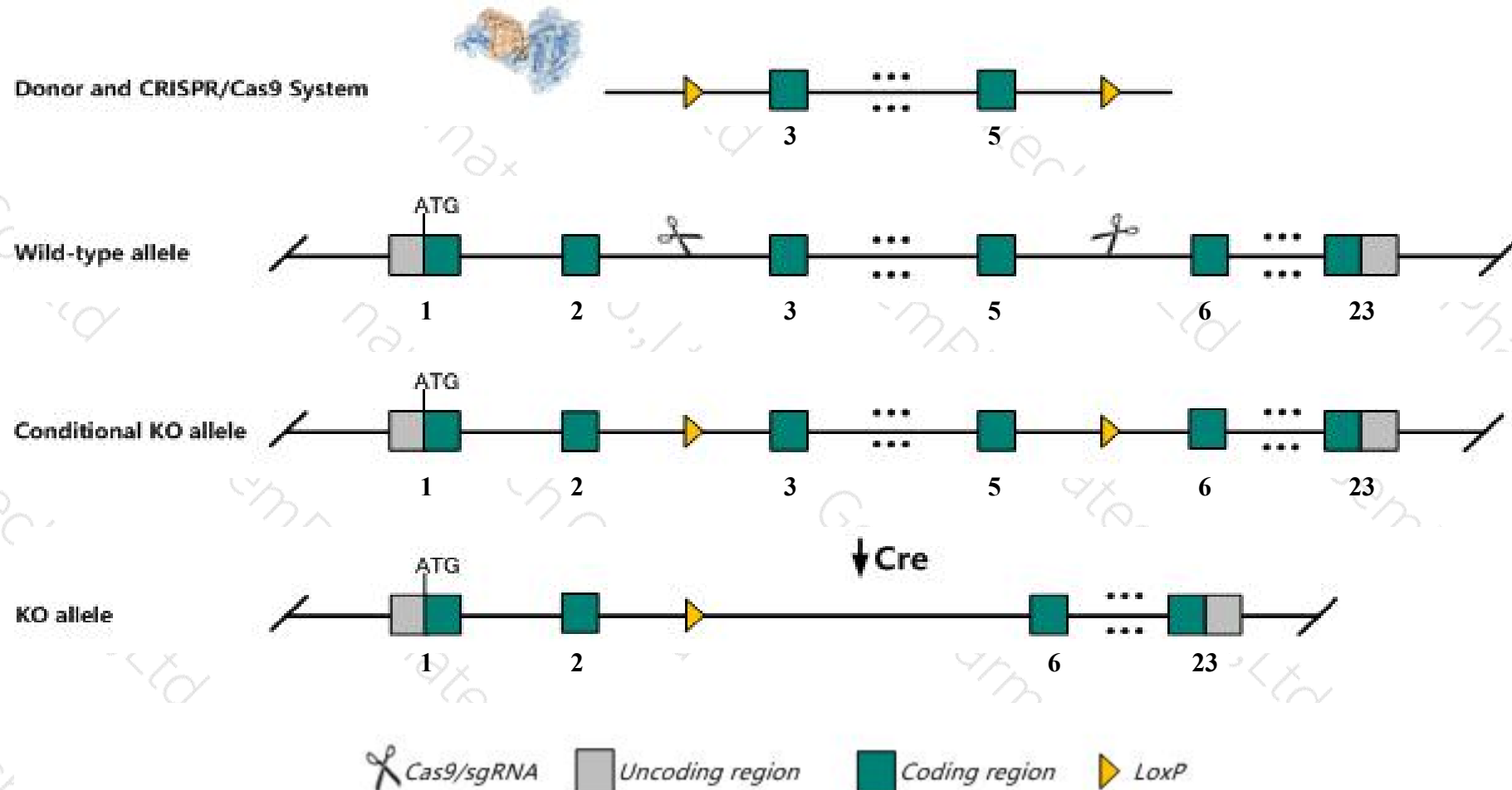|	
Project Name	*Fsip2*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

This model will use CRISPR/Cas9 technology to edit the *Fsip2* gene. The schematic diagram is as follows:

Technical routes

➤ The *Fsip2* gene has 3 transcripts. According to the structure of *Fsip2* gene, exon3-exon5 of *Fsip2-203*(ENSMUST00000143764.8) transcript is recommended as the knockout region.The region contains 392bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Fsip2* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA and Donor 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.

➤ The flox mice was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

➢ Transcript *Fsip2*-202&203 may not be affected.

➢ The *Fsip2* gene is located on the Chr2. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

Fsip2   fibrous sheath-interacting protein 2 [*Mus musculus* (house mouse)]

Gene ID: 241516, updated on 26-Jun-2020

▲ Summary

Official Symbol	Fsip2 provided by MGI
Official Full Name	fibrous sheath-interacting protein 2 provided by MGI
Primary source	MGI:MGI:2664111
See related	Ensembl:ENSMUSG00000075249
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Restricted expression toward testis adult (RPKM 3.1) See more
Orthologs	human all

▲ Genomic context

Location: 2; 2 D   See Fsip2 in Genome Data Viewer

Exon count: 23

| Annotation release	Status	Assembly	Chr	Location
108.20200622	current	GRCm38.p6 (GCF_000001635.26)	2	NC_000068.7 (82943634..83008937)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	2	NC_000068.6 (82783797..82849094)

Transcript information（Ensembl）

The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fsip2-203	ENSMUST00000143764.8	21116	6995aa	Protein coding	-	A2ARZ3	TSL:5 GENCODE basic APPRIS P1
Fsip2-201	ENSMUST00000132967.1	3932	1009aa	Nonsense mediated decay	-	F7BGZ6	CDS 5' incomplete TSL:5
Fsip2-202	ENSMUST00000136202.1	740	135aa	Nonsense mediated decay	-	F6ZQl5	CDS 5' incomplete TSL:5

The strategy is based on the design of *Fsip2-203* transcript,the transcription is shown below:
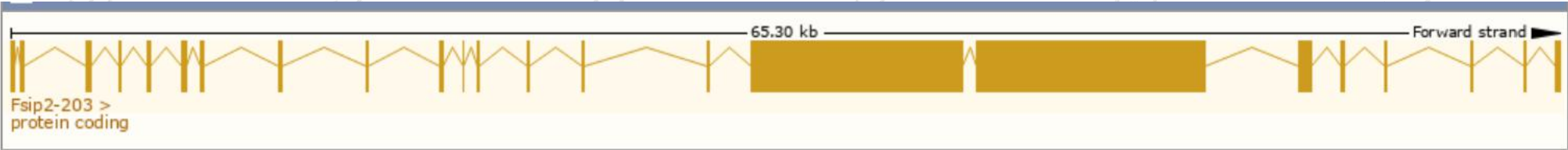
Genomic location distribution
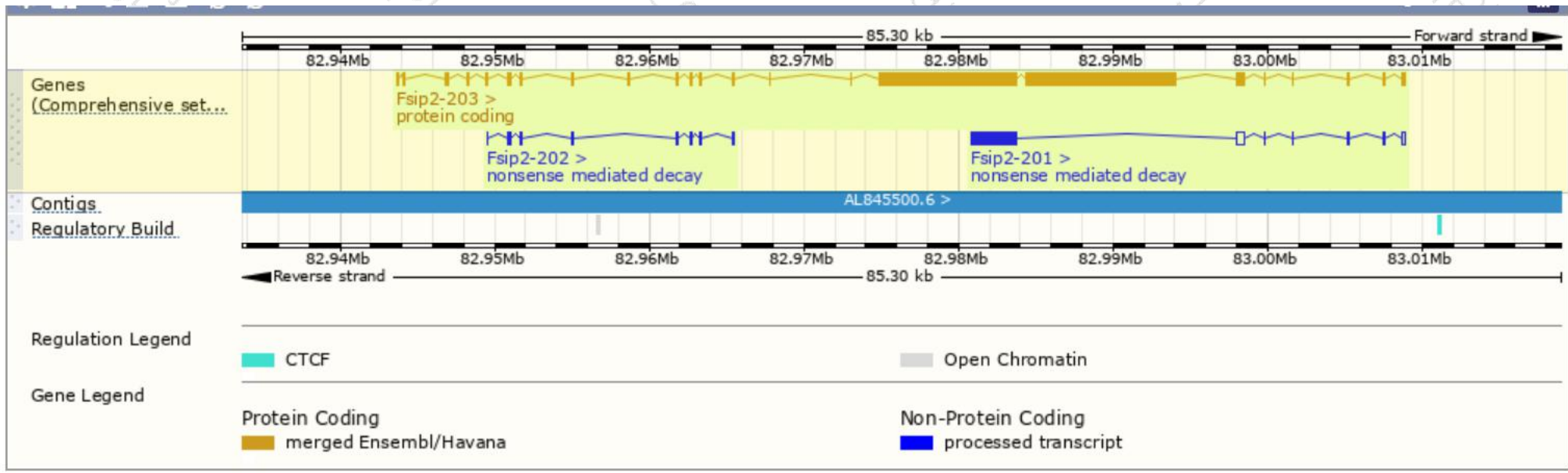
Protein domain

If you have any questions, you are welcome to inquire.
Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd