Actn1 Cas9-CKO Strategy

Designer: Yang Zeng
Reviewer: Yanhua Shen

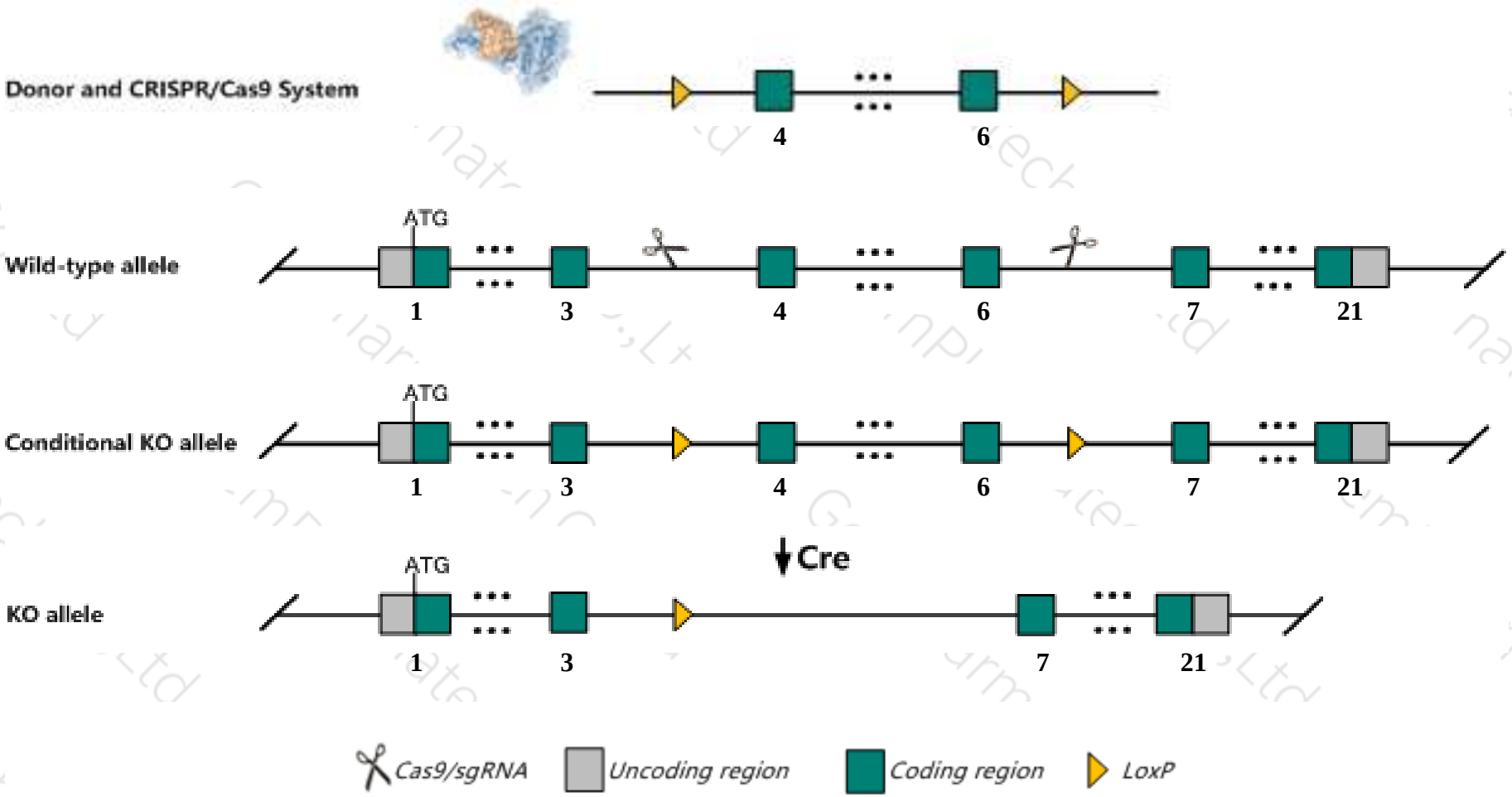
Design Date: 2019-11-26

Project Overview

Project Name	***Actn1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Actn1* gene. The schematic diagram is as follows:



Technical routes

- The *Actn1* gene has 7 transcripts. According to the structure of *Actn1* gene, exon4-exon6 of *Actn1-201* (ENSMUST00000021554.15) transcript is recommended as the knockout region.The region contains 254bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Actn1* 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

➢The Floxed region is close to *Gm47765* gene.Knockout the region may affect the function of *Gm47765* gene.

➢ The *Actn1* gene is located on the Chr12. 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）

集萃药康 GemPharmatech

Actn1 actinin, alpha 1 [*Mus musculus* (house mouse)]

Gene ID: 109711, updated on 28-Oct-2019

Summary

Official Symbol	Actn1 provided by MGI
Official Full Name	actinin, alpha 1 provided by MGI
Primary source	MGI:MGI:2137706
See related	Ensembl:ENSMUSG00000015143
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
Also known as	Actn1a; 3110023F10Rik
Expression	Broad expression in bladder adult (RPKM 126.9), ovary adult (RPKM 50.7) and 22 other tissues See more
Orthologs	human all

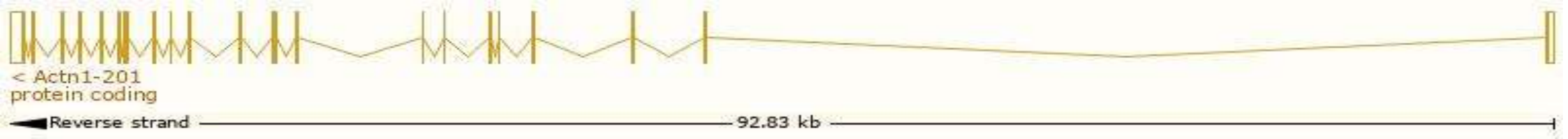
Chromosome 12 - NC_000078.6

Transcript information（Ensembl）

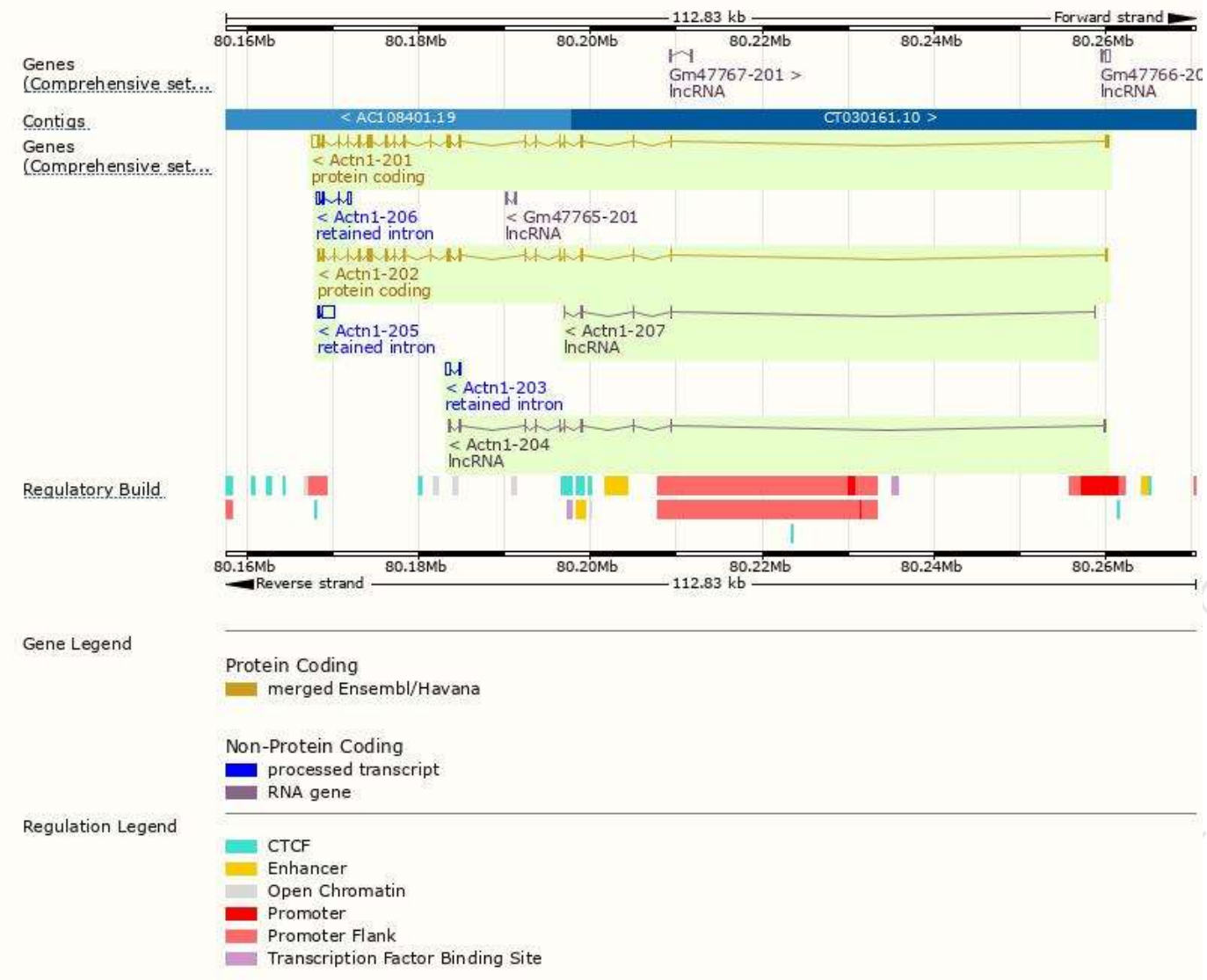
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Actn1-201	ENSMUST00000021554.15	3734	892aa	Protein coding	CCDS26011	Q7TPR4	TSL:1 GENCODE basic APPRIS P1
Actn1-202	ENSMUST00000167327.1	2664	887aa	Protein coding	CCDS83972	A1BN54	TSL:1 GENCODE basic
Actn1-205	ENSMUST00000219382.1	1410	No protein	Retained intron	-	-	TSL:1
Actn1-206	ENSMUST00000219634.1	1034	No protein	Retained intron	-	-	TSL:1
Actn1-203	ENSMUST00000217984.1	449	No protein	Retained intron	-	-	TSL:3
Actn1-204	ENSMUST00000218874.1	943	No protein	lncRNA	-	-	TSL:5
Actn1-207	ENSMUST00000220351.1	505	No protein	lncRNA	-	-	TSL:3

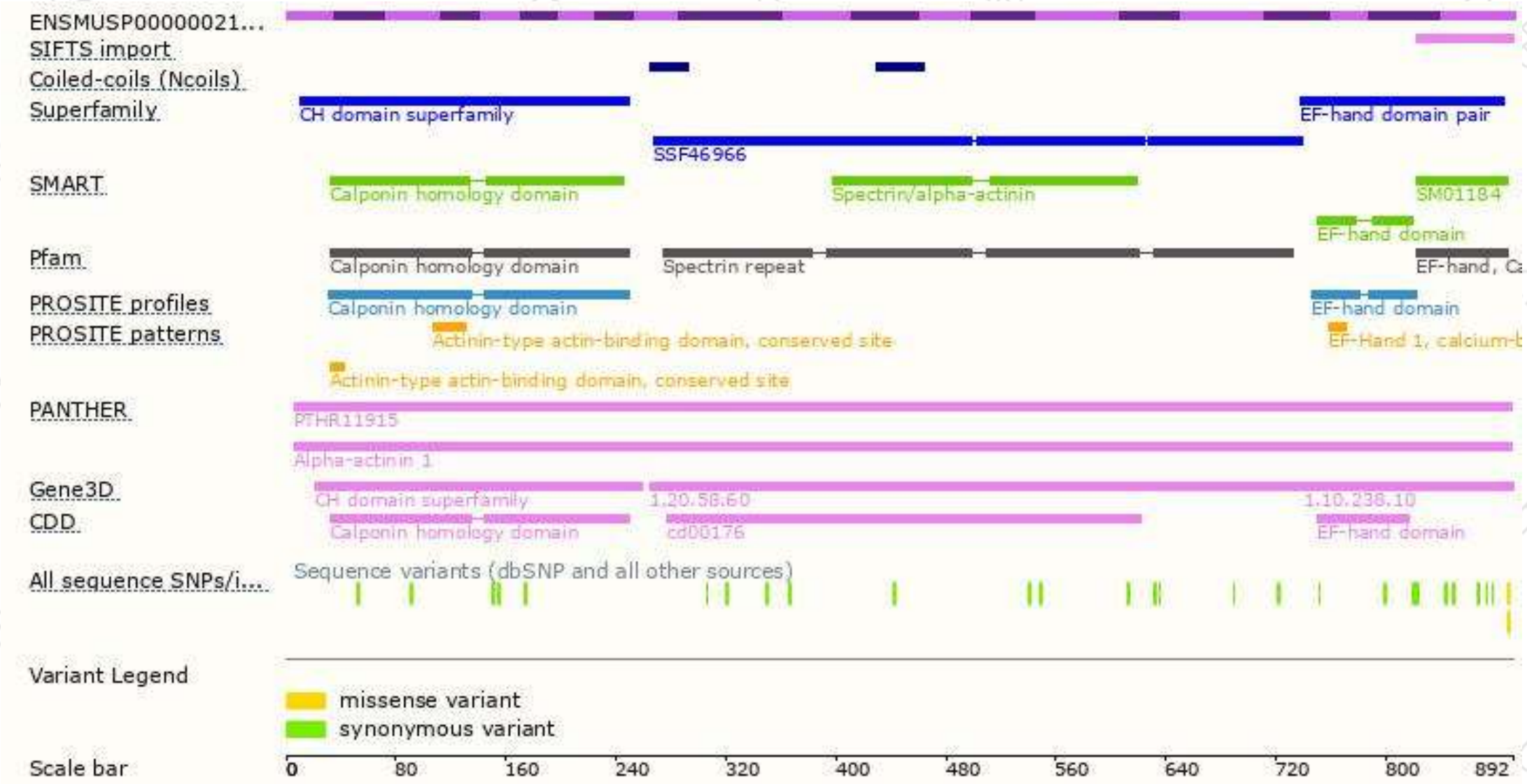
The strategy is based on the design of *Actn1-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



集萃药康
GemPharmatech

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