

Actn4 Cas9-CKO Strategy

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Project Overview

Project Name

Actn4

Project type

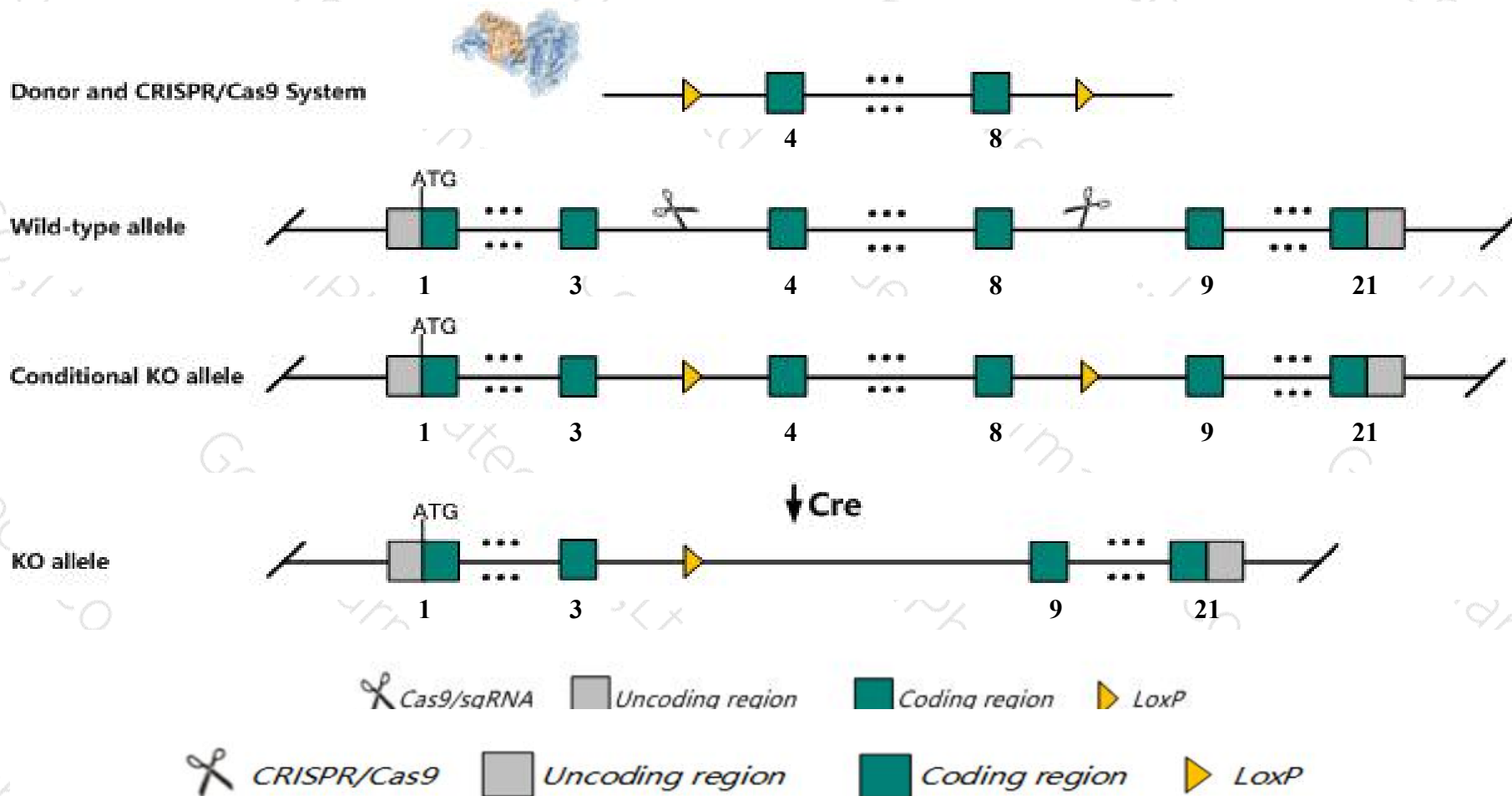
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Actn4* gene. The schematic diagram is as follows:



Technical routes

- The *Actn4* gene has 11 transcripts. According to the structure of *Actn4* gene, exon4-exon8 of *Actn4-201* (ENSMUST00000068045.13) transcript is recommended as the knockout region. The region contains 422bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Actn4* gene. The brief process is as follows: CRISPR/Cas9 system 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 will be 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.

- According to the existing MGI data, mice homozygous for a disruption in this gene die either around birth or within a few months of birth. Those who do survive after birth show poor growth and kidney abnormalities including glomerulosclerosis. This is manifested functionally as proteinuria and abnormal blood urea nitrogen.
- The *Actn4* gene is located on the Chr7. 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)

Actn4 actinin alpha 4 [Mus musculus (house mouse)]

Gene ID: 60595, updated on 13-Mar-2020

Summary



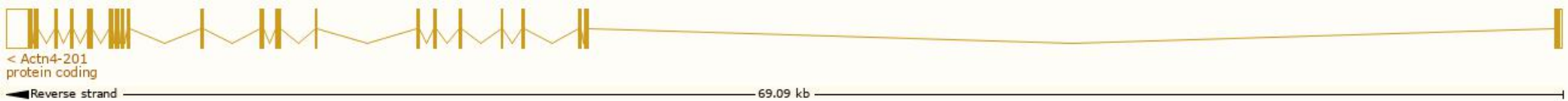
Official Symbol	Actn4 provided by MGI
Official Full Name	actinin alpha 4 provided by MGI
Primary source	MGI:MGI:1890773
See related	Ensembl:ENSMUSG00000054808
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C77391
Expression	Ubiquitous expression in small intestine adult (RPKM 213.0), large intestine adult (RPKM 207.4) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

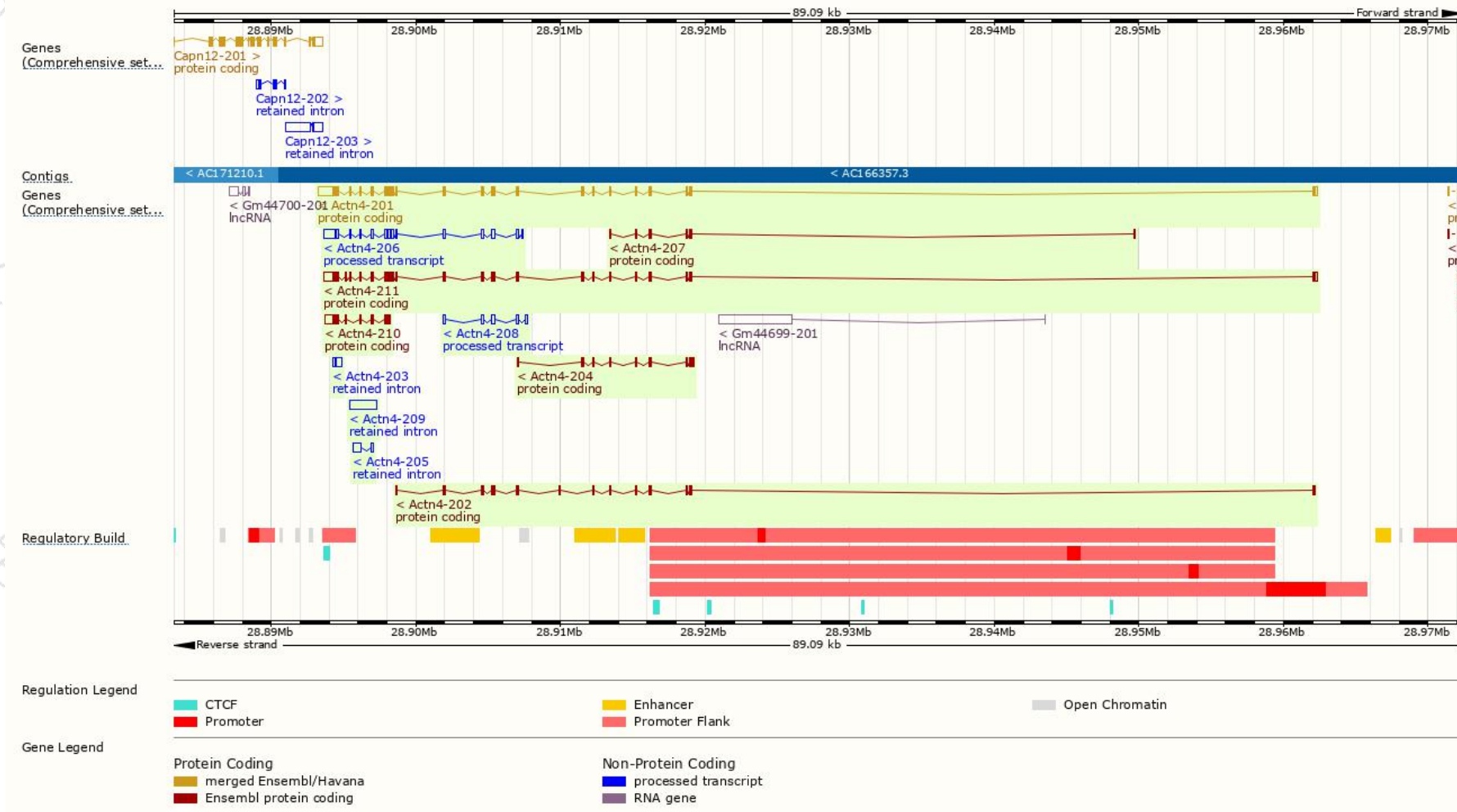
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Actn4-201	ENSMUST00000068045.13	3877	912aa	Protein coding	CCDS21061	P57780 Q3ULT2	TSL:1 GENCODE basic APPRIS P2
Actn4-211	ENSMUST00000217157.1	3530	932aa	Protein coding	-	A0A1L1SV25	TSL:5 GENCODE basic APPRIS ALT1
Actn4-202	ENSMUST00000127210.7	1594	518aa	Protein coding	-	E9Q2W9	CDS 3' incomplete TSL:5
Actn4-210	ENSMUST00000216863.1	1489	323aa	Protein coding	-	A0A1L1SVJ6	CDS 5' incomplete TSL:5
Actn4-204	ENSMUST00000140622.7	774	206aa	Protein coding	-	D3Z0L8	CDS 3' incomplete TSL:5
Actn4-207	ENSMUST00000148196.2	617	133aa	Protein coding	-	D3Z761	CDS 3' incomplete TSL:5
Actn4-206	ENSMUST00000144909.7	2581	No protein	Processed transcript	-	-	TSL:1
Actn4-208	ENSMUST00000150493.1	726	No protein	Processed transcript	-	-	TSL:3
Actn4-209	ENSMUST00000207765.1	1890	No protein	Retained intron	-	-	TSL:NA
Actn4-205	ENSMUST00000143584.1	750	No protein	Retained intron	-	-	TSL:1
Actn4-203	ENSMUST00000129338.1	508	No protein	Retained intron	-	-	TSL:1

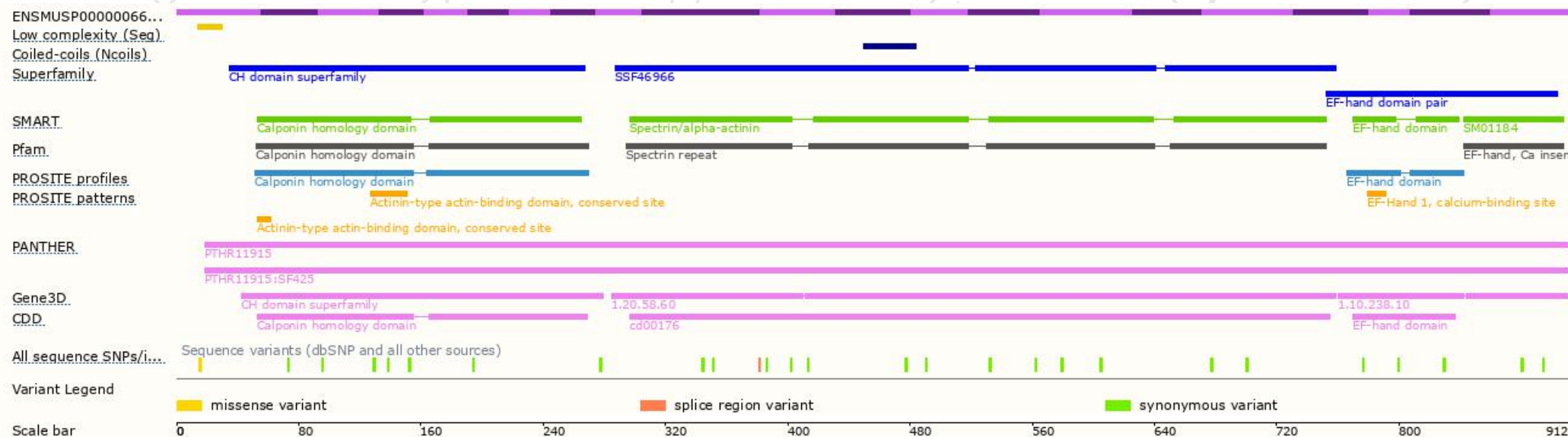
The strategy is based on the design of *Actn4-201* transcript, the transcription is shown below



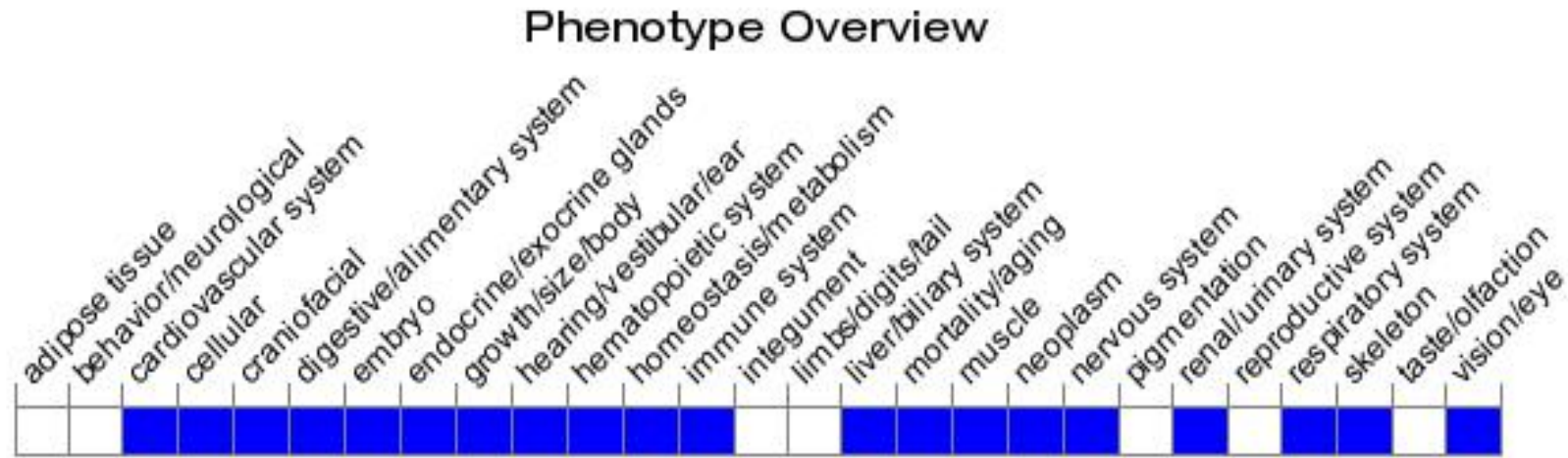
Genomic location distribution



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/>).

According to the existing MGI data, mice homozygous for a disruption in this gene die either around birth or within a few months of birth. Those who do survive after birth show poor growth and kidney abnormalities including glomerulosclerosis. This is manifested functionally as proteinuria and abnormal blood urea nitrogen.

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

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