Rnf4 Cas9-CKO Strategy

Designer: Huimin Su

Reviewer: Ruiuri Zhang

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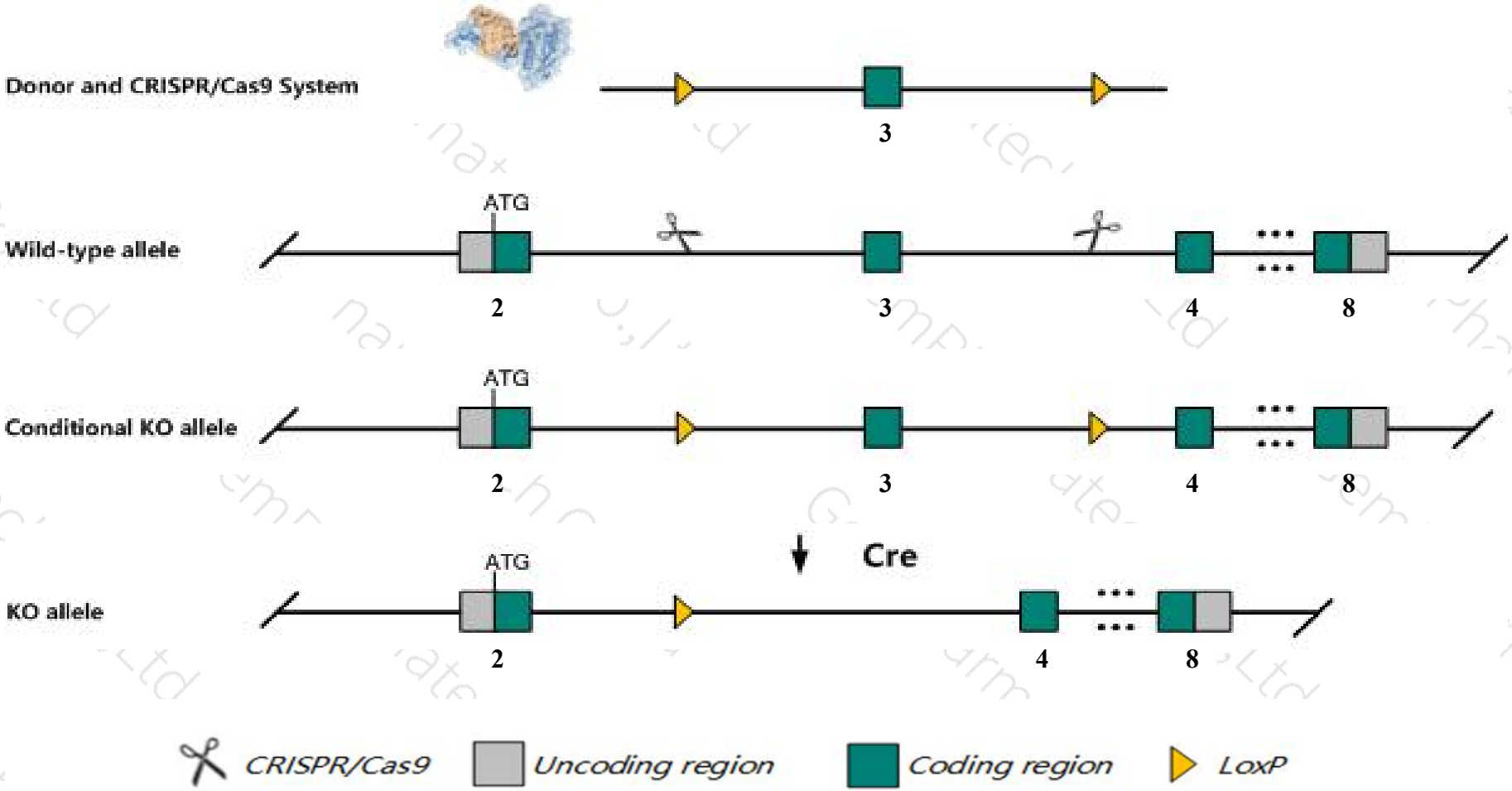
Project Overview

Project Name	***Rnf4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rnf4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Rnf4* gene has 12 transcripts. According to the structure of *Rnf4* gene, exon3 of *Rnf4-207*(ENSMUST00000182709.7) transcript is recommended as the knockout region.The region contains 127bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Rnf4* 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.

Notice

➢ According to the existing MGI data,mice homozygous for a null mutation display lethality throughout fetal growth and development with ventricular septal defects and cardiac insufficiency. MEFs from homozygous mice display global DNA hypermethylation.

➢ The *Rnf4* gene is located on the Chr5. 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）

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Rnf4 ring finger protein 4 [Mus musculus (house mouse)]

Gene ID: 19822, updated on 13-Mar-2020

Summary

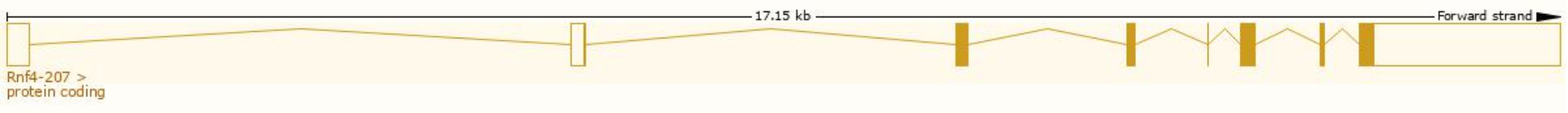
Official Symbol	Rnf4 provided by MGI
Official Full Name	ring finger protein 4 provided byMGI
Primary source	MGI:MGI:1201691
See related	Ensembl:ENSMUSG00000029110
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AU018689, Gtrgeo8, SNURF
Summary	This gene encodes a small nuclear RING finger protein that mediates ubiquitylation of polysumoylated proteins. Deficiency of the encoded protein in mice leads to embryonic lethality and global DNA hypermethylation. A similar protein in humans is required for arsenic-induced degradation of promyelocytic leukemia protein in acute promyelocytic leukemia. Alternative splicing of this gene results in multiple transcript variants. A pseudogene for this gene has been identified on chromosome 10. [provided by RefSeq, Jan 2015]
Expression	Ubiquitous expression in placenta adult (RPKM 51.2), CNS E11.5 (RPKM 42.5) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

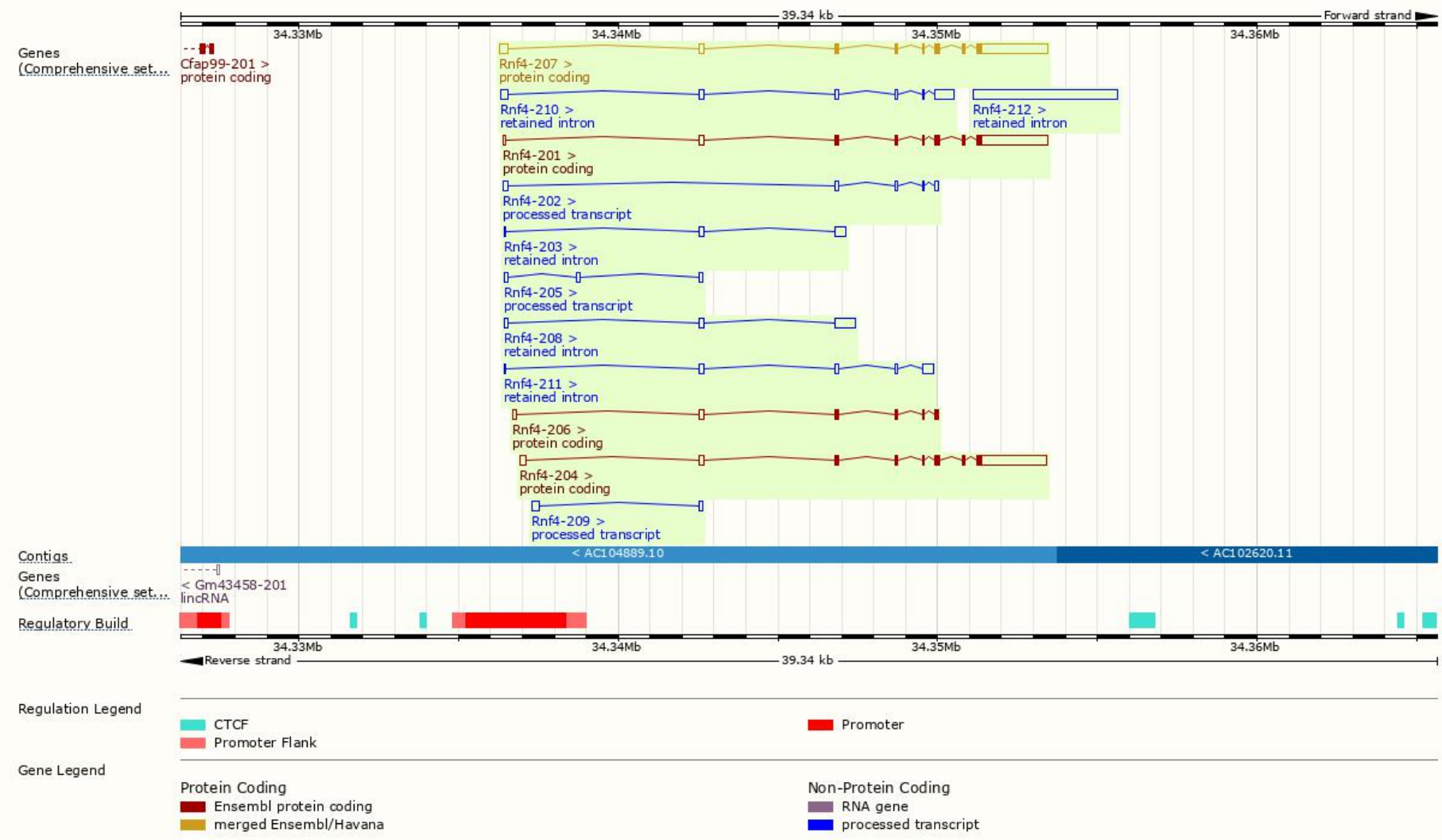
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein ▲	Biotype	CCDS	UniProt	Flags
Rnf4-206	ENSMUST00000182583.7	619	115aa	Protein coding	-	S4R2C9	CDS 3' incomplete TSL:5
Rnf4-207	ENSMUST00000182709.7	3046	194aa	Protein coding	CCDS57336	Q9QZS2	TSL:1 GENCODE basic APPRIS P1
Rnf4-204	ENSMUST00000182047.1	2944	194aa	Protein coding	CCDS57336	Q9QZS2	TSL:1 GENCODE basic APPRIS P1
Rnf4-201	ENSMUST00000030992.12	2895	194aa	Protein coding	CCDS57336	Q9QZS2	TSL:1 GENCODE basic APPRIS P1
Rnf4-202	ENSMUST00000181991.7	491	No protein	Processed transcript	-	-	TSL:2
Rnf4-205	ENSMUST00000182582.1	371	No protein	Processed transcript	-	-	TSL:3
Rnf4-209	ENSMUST00000182810.1	357	No protein	Processed transcript	-	-	TSL:3
Rnf4-212	ENSMUST00000183265.1	4514	No protein	Retained intron	-	-	TSL:NA
Rnf4-210	ENSMUST00000182970.7	1205	No protein	Retained intron	-	-	TSL:2
Rnf4-208	ENSMUST00000182772.7	928	No protein	Retained intron	-	-	TSL:1
Rnf4-211	ENSMUST00000183071.1	723	No protein	Retained intron	-	-	TSL:3
Rnf4-203	ENSMUST00000181999.7	586	No protein	Retained intron	-	-	TSL:2

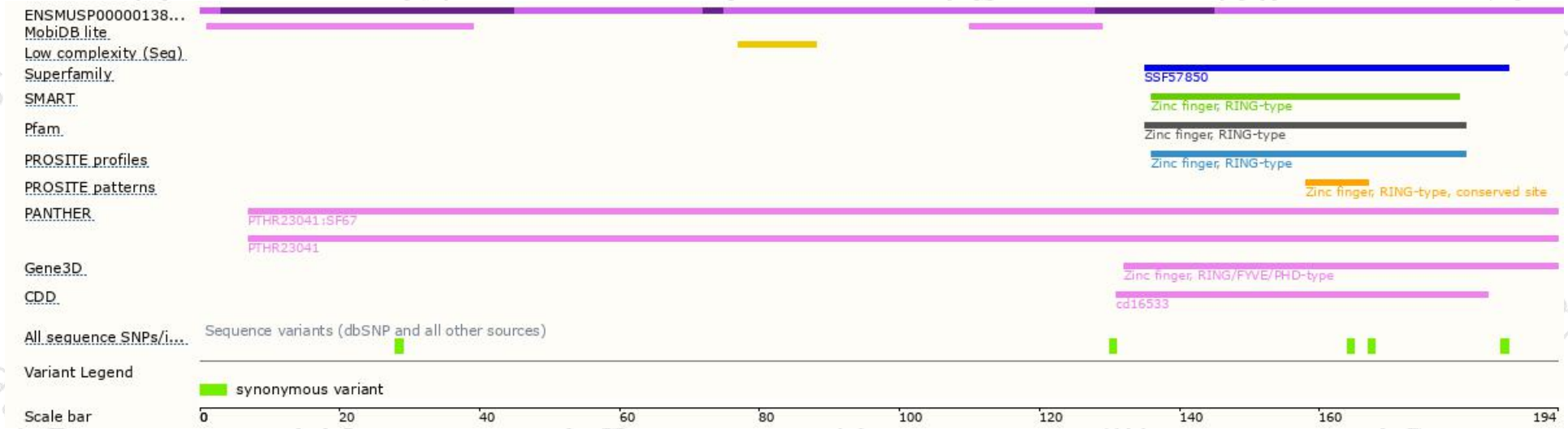
The strategy is based on the design of *Rnf4-207* transcript,the transcription is shown below:



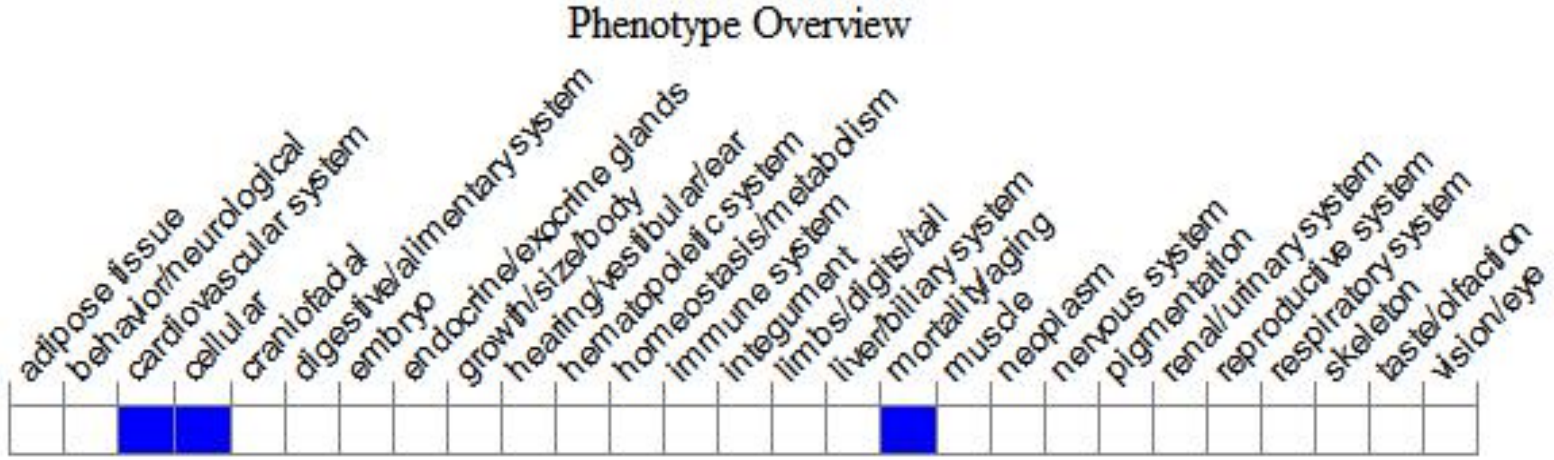
Genomic location distribution



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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集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd