Rnf5 Cas9-CKO Strategy

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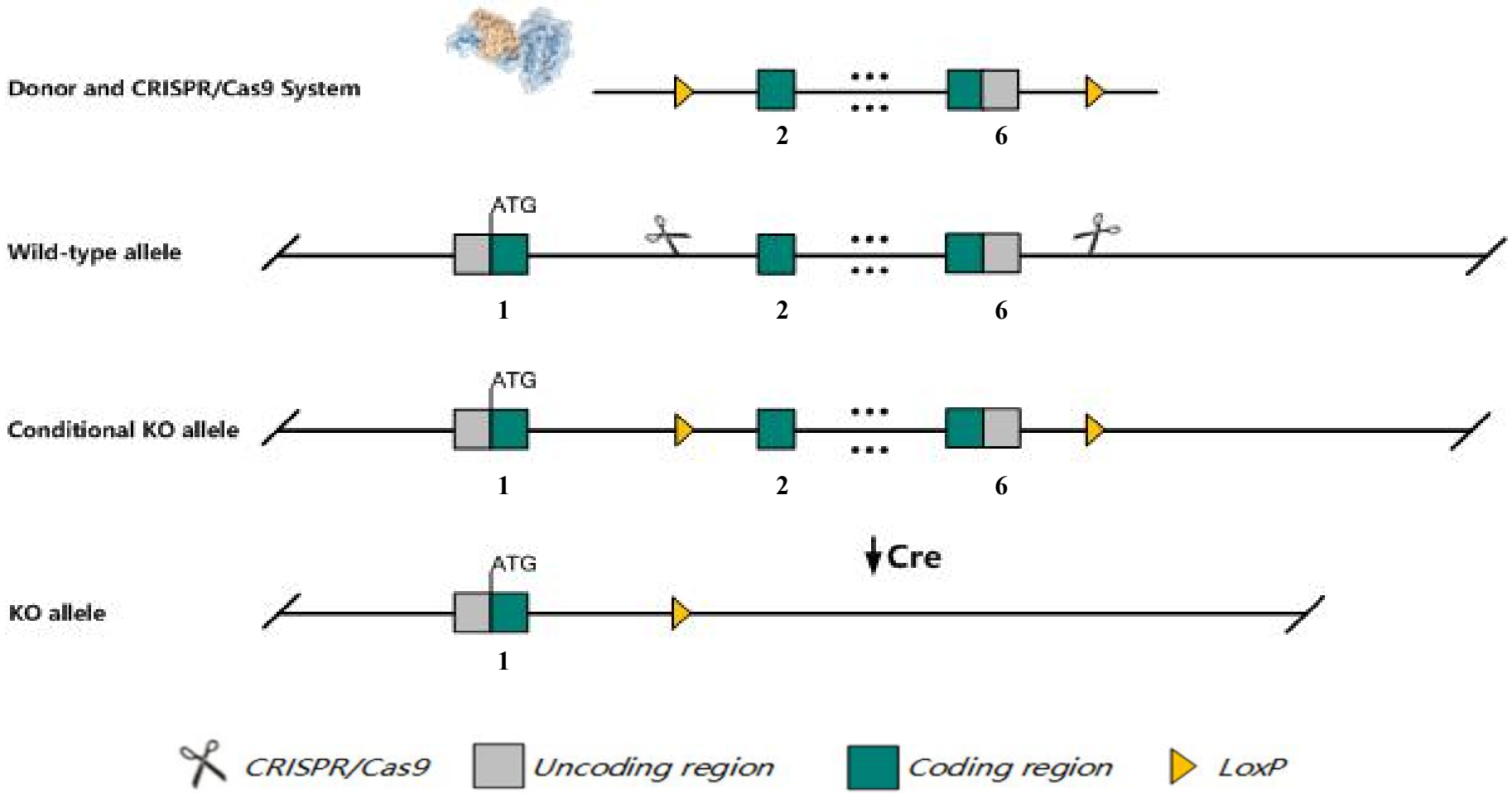
Project Overview

Project Name	***Rnf5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rnf5* gene. The schematic diagram is as follows:



Technical routes

The *Rnf5* gene has 3 transcripts. According to the structure of *Rnf5* gene, exon2-exon6 of *Rnf5-201*(ENSMUST00000015622.7) transcript is recommended as the knockout region.The region contains 403bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Rnf5* 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,when subjected to muscle damage by cardiotoxin treatment, mice homozygous for a targeted null mutation display attenuated muscle regeneration associated with a delayed ER stress response.

Knockout the region may affect the function of Ager gene.

The *Rnf5* gene is located on the Chr17. 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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Rnf5 ring finger protein 5 [Mus musculus (house mouse)]

Gene ID: 54197, updated on 13-Mar-2020

Summary

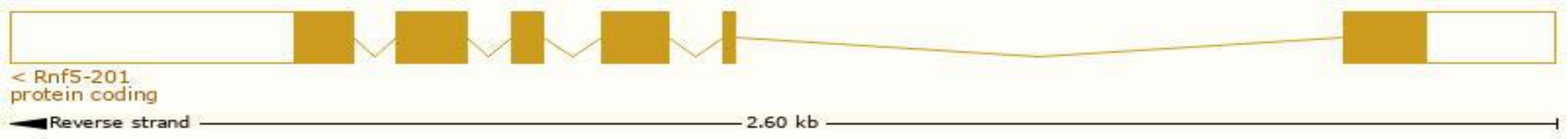
Official Symbol	Rnf5 provided by MGI
Official Full Name	ring finger protein 5 provided byMGI
Primary source	MGI:MGI:1860076
See related	Ensembl:ENSMUSG00000015478
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2410131O05Rik, AA407576, NG2
Expression	Ubiquitous expression in kidney adult (RPKM 88.4), large intestine adult (RPKM 73.5) and 28 other tissuesSee more
Orthologs	human all

Transcript information Ensembl

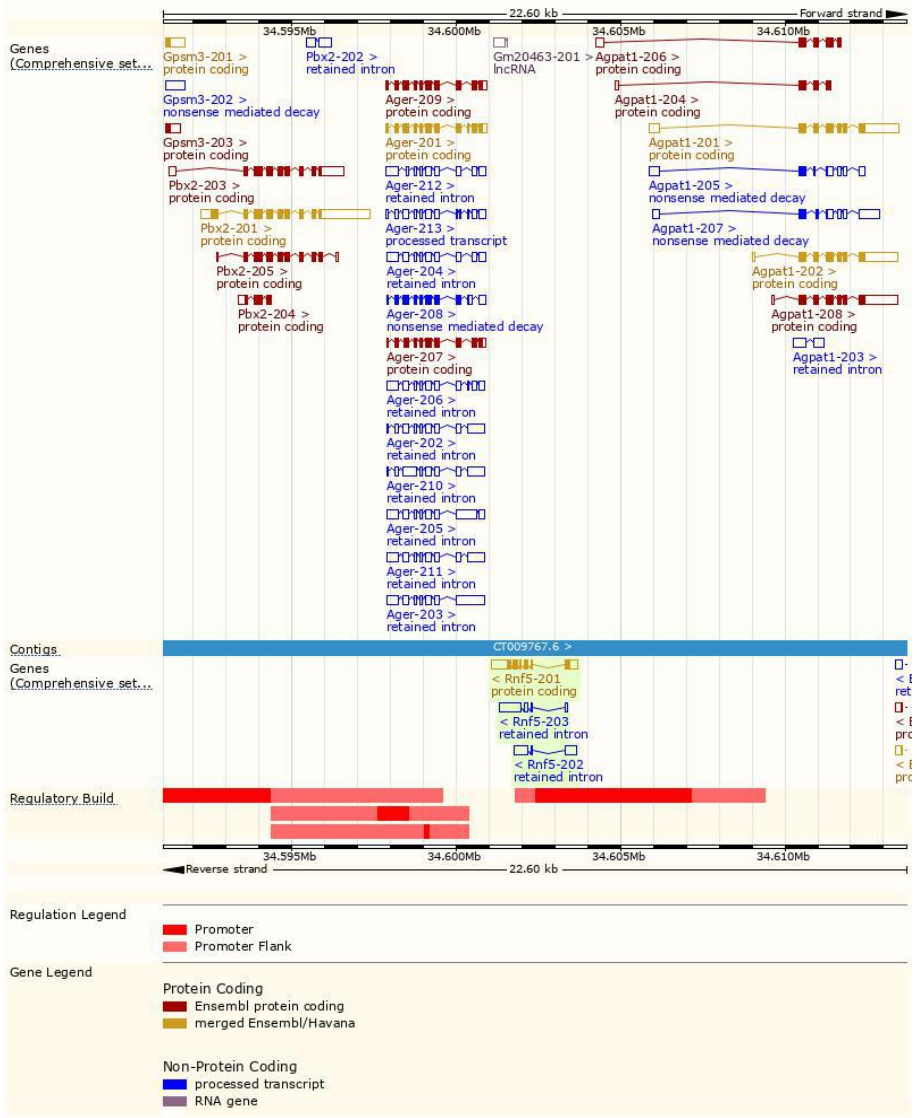
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rnf5-201	ENSMUST00000015622.7	1238	180aa	Protein coding	CCDS28650	O35445	TSL:1 GENCODE basic APPRIS P1
Rnf5-203	ENSMUST00000174475.7	850	No protein	Retained intron	-	-	TSL:2
Rnf5-202	ENSMUST00000174045.1	763	No protein	Retained intron	-	-	TSL:2

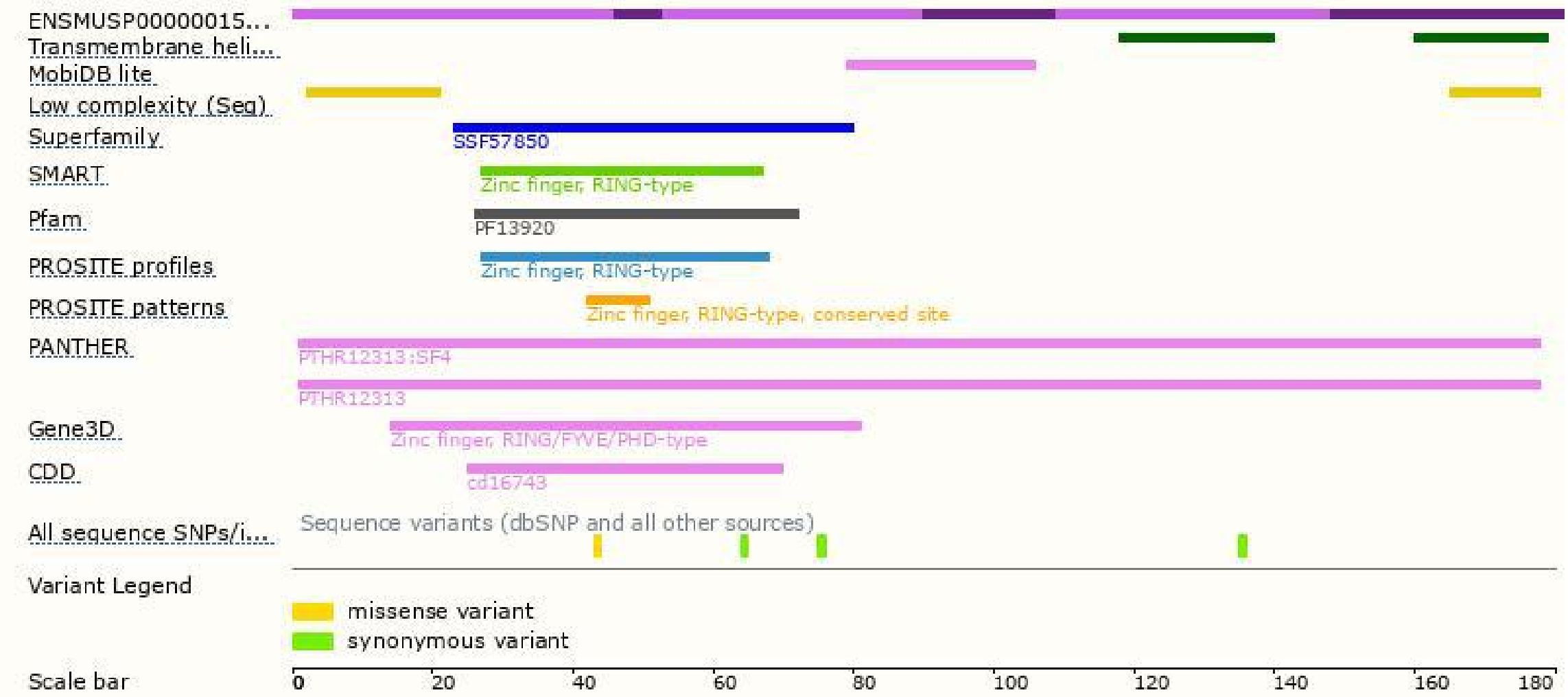
The strategy is based on the design of *Rnf5-201* transcript,the transcription is shown below:



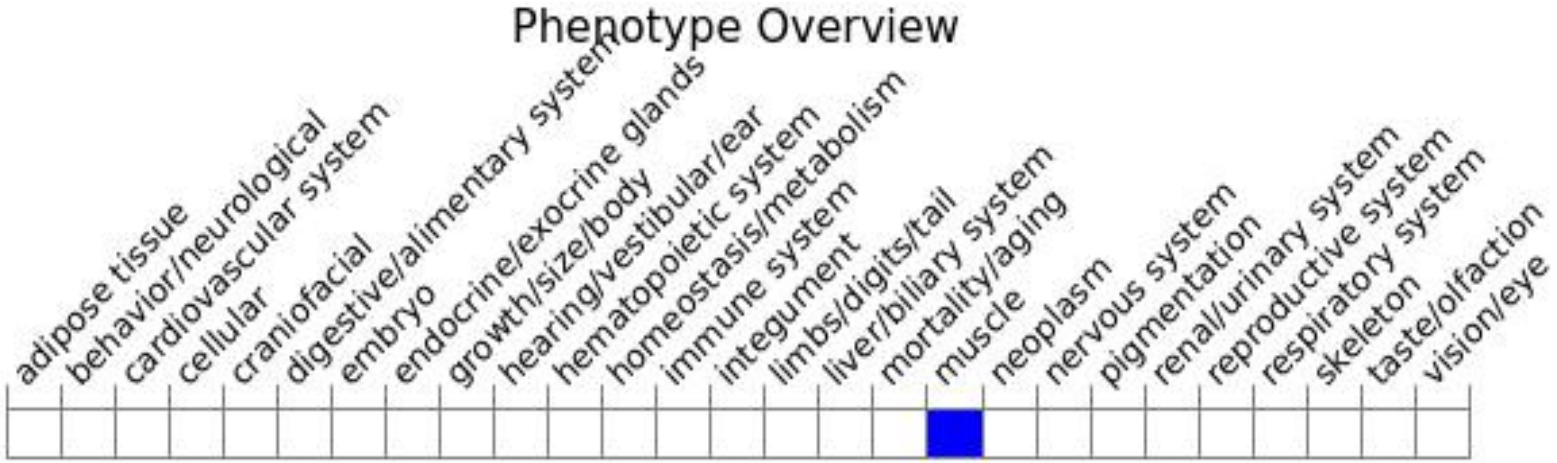
Genomic location distribution



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,when subjected to muscle damage by cardiotoxin treatment, mice homozygous for a targeted null mutation display attenuated muscle regeneration associated with a delayed ER stress response.

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