Ryr1 Cas9-CKO Strategy

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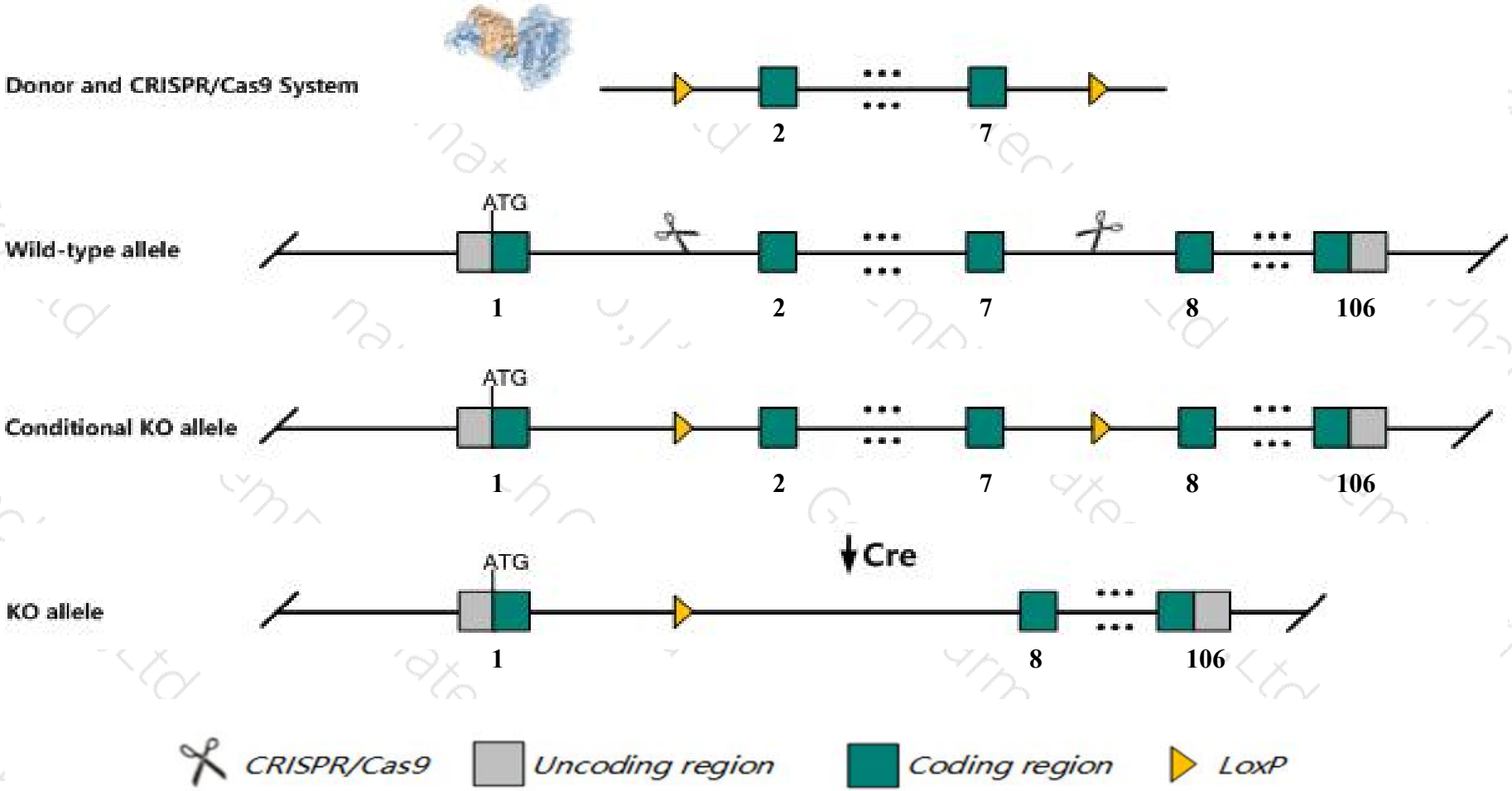
Project Overview

Project Name	***Ryr1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ryr1* gene. The schematic diagram is as follows:



Technical routes

- The *Ryr1* gene has 8 transcripts. According to the structure of *Ryr1* gene, exon2-exon7 of *Ryr1-202* (ENSMUST00000179893.8) transcript is recommended as the knockout region.The region contains 586bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ryr1* 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,homozygotes for a targeted null mutation and a similar enu-induced mutation are born with a rounded body shape, edema, thin and misshapened ribs, and abnormal muscle fibers. mutants die perinatally.

- The *Ryr1* 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）

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Ryr1 ryanodine receptor 1, skeletal muscle [Mus musculus (house mouse)]

Gene ID: 20190, updated on 15-Mar-2020

Summary

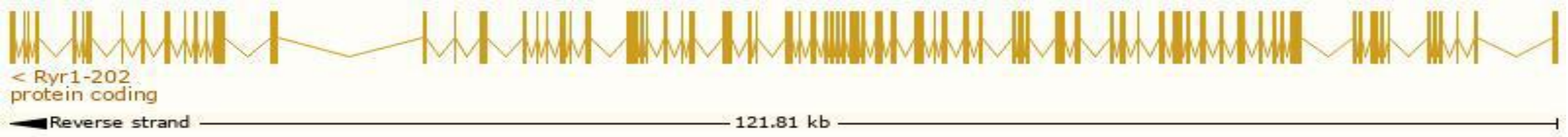
Official Symbol	Ryr1 provided by MGI
Official Full Name	ryanodine receptor 1, skeletal muscle provided by MGI
Primary source	MGI:MGI:99659
See related	Ensembl:ENSMUSG00000030592
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI528790, RYR-1, Ryr, skrr
Expression	Biased expression in mammary gland adult (RPKM 14.4), limb E14.5 (RPKM 5.1) and 8 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

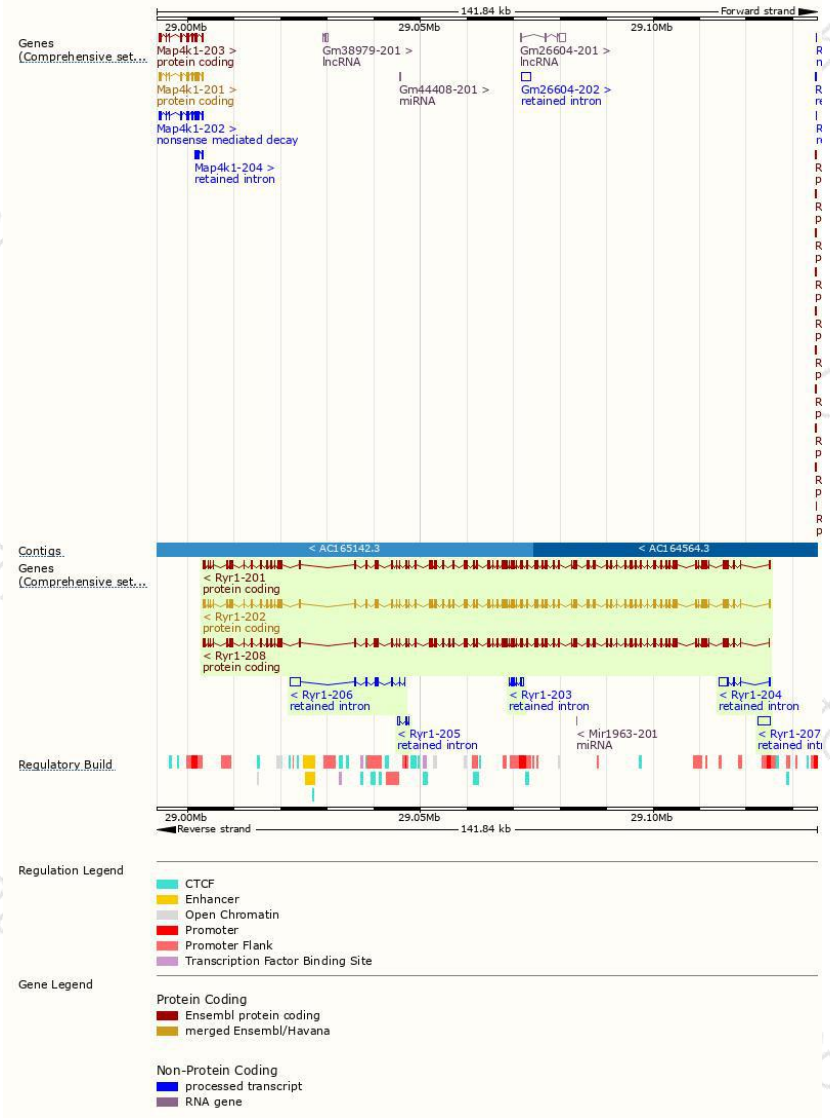
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ryr1-202	ENSMUST00000179893.8	15358	5035aa	Protein coding	CCDS39866	E9PZQ0	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2
Ryr1-201	ENSMUST00000032813.9	15352	5033aa	Protein coding	-	K3W4M2	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Ryr1-208	ENSMUST00000214374.1	15300	5061aa	Protein coding	-	A0A1L1SQG7	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Ryr1-206	ENSMUST00000208318.1	3044	No protein	Retained intron	-	-	TSL:2
Ryr1-207	ENSMUST00000208922.1	2525	No protein	Retained intron	-	-	TSL:NA
Ryr1-204	ENSMUST00000207783.1	2366	No protein	Retained intron	-	-	TSL:2
Ryr1-203	ENSMUST00000207764.1	1028	No protein	Retained intron	-	-	TSL:5
Ryr1-205	ENSMUST00000208010.1	698	No protein	Retained intron	-	-	TSL:3

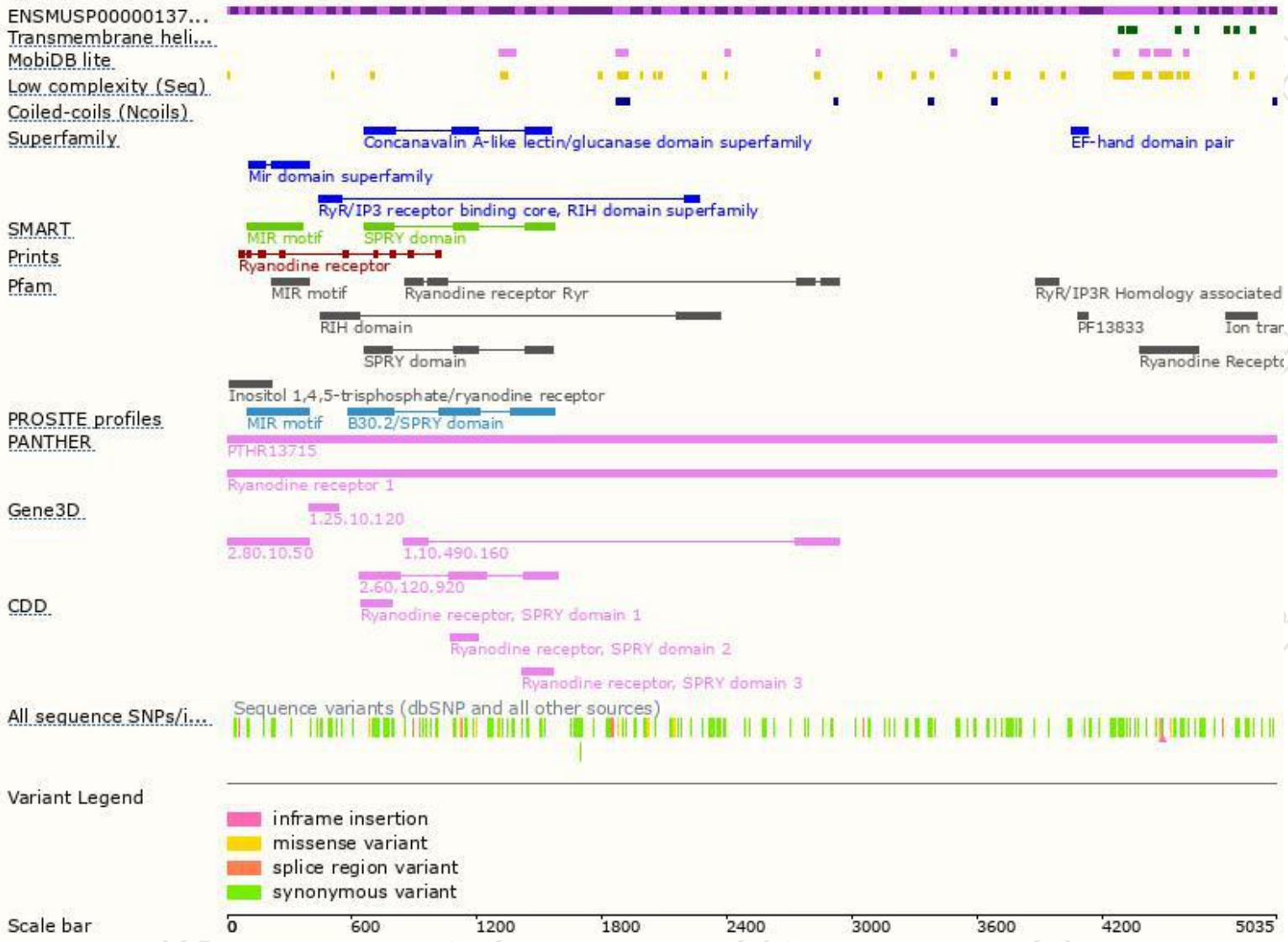
The strategy is based on the design of *Ryr1-202* transcript,the transcription is shown below:



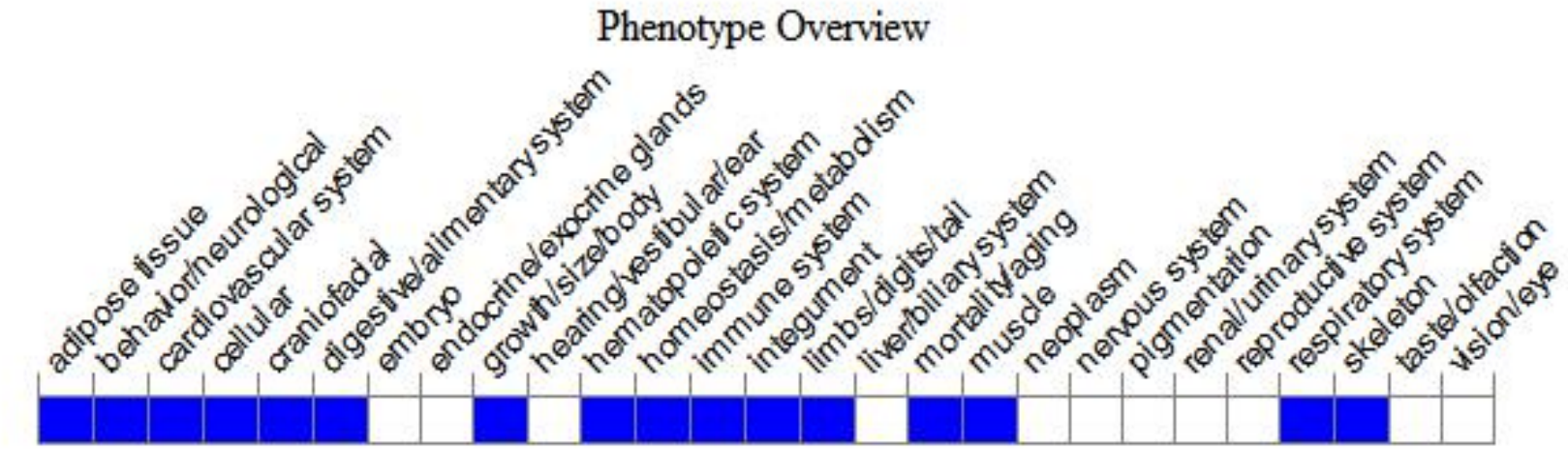
Genomic location distribution



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

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