Ryr2 Cas9-KO Strategy

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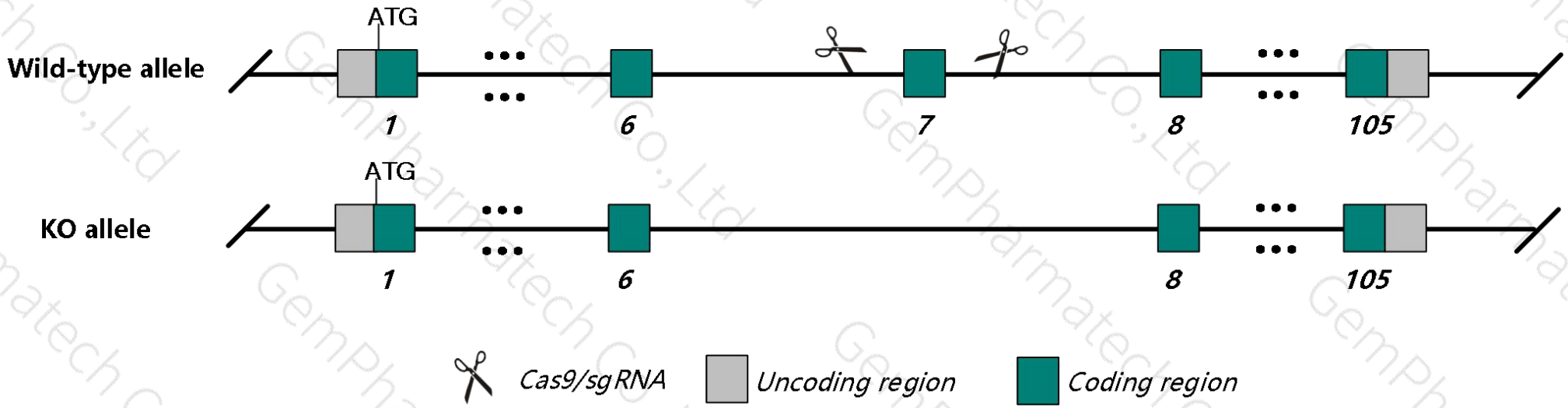
Project Overview

Project Name	***Ryr2***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ryr2* gene. The schematic diagram is as follows:



Technical routes

- The *Ryr2* gene has 14 transcripts. According to the structure of *Ryr2* gene, exon7 of *Ryr2-201* (ENSMUST00000021750.14) transcript is recommended as the knockout region.The region contains 79bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ryr2* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice

- According to the existing MGI data,Homozygous null mice show embryonic lethality during organogenesis and altered cardiomyocyte morphology. Homozygotes for a phosphorylation defective allele show decreased susceptibility to myocardial infarction-induced heart failure. Homozygotes for the R420W allele show lymphoid organ hypertrophy.
- 127 amino acids remain at the N-terminus and some functions may be retained.
- Transcript *Ryr2*-204,205,206,207,208~214 may be unaffected.
- The *Ryr2* gene is located on the Chr13. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Ryr2 ryanodine receptor 2, cardiac [*Mus musculus* (house mouse)]

Gene ID: 20191, updated on 7-May-2019

Summary

Official Symbol	Ryr2 provided by MGI
Official Full Name	ryanodine receptor 2, cardiac provided by MGI
Primary source	MGI:MGI:99685
See related	Ensembl:ENSMUSG00000021313
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	RYR-2; 9330127I20Rik
Expression	Biased expression in heart adult (RPKM 43.4), cortex adult (RPKM 7.6) and 3 other tissues See more
Orthologs	human all

Genomic context

Location: 13 A1; 13 4.38 cM

See Ryr2 in Genome Data Viewer

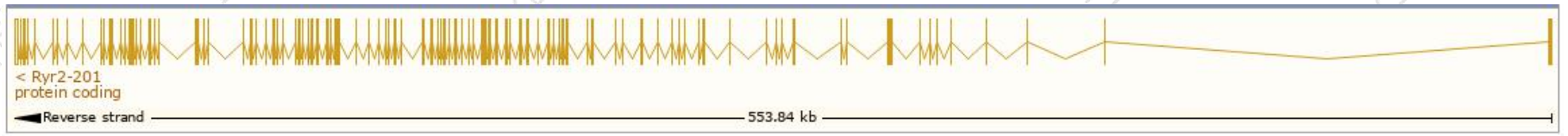
Exon count: 109

Transcript information（Ensembl）

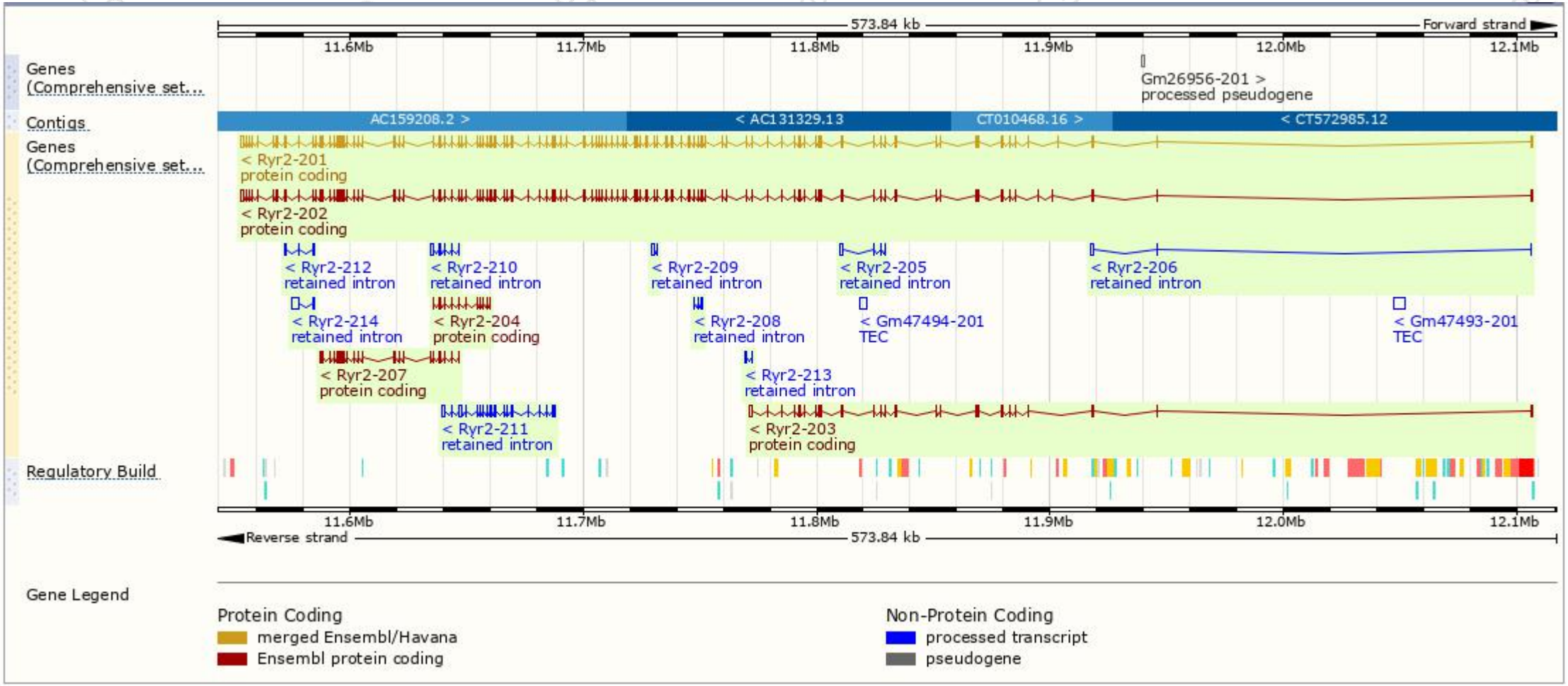
The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ryr2-201	ENSMUST00000021750.14	16814	4966aa	Protein coding	CCDS49206	E9Q401	TSL:1 GENCODE basic APPRIS P2
Ryr2-202	ENSMUST00000170156.2	16813	4966aa	Protein coding	-	F6U7V1	TSL:5 GENCODE basic APPRIS ALT2
Ryr2-203	ENSMUST00000220597.1	4924	1020aa	Protein coding	-	Q80ZZ5	TSL:1 GENCODE basic
Ryr2-207	ENSMUST00000221527.1	3086	1028aa	Protein coding	-	A0A1Y7VJP1	CDS 5' and 3' incomplete TSL:5
Ryr2-204	ENSMUST00000220712.1	1106	369aa	Protein coding	-	A0A1Y7VK09	CDS 5' and 3' incomplete TSL:5
Ryr2-211	ENSMUST00000221941.1	4117	No protein	Retained intron	-	-	TSL:5
Ryr2-214	ENSMUST00000222788.1	3794	No protein	Retained intron	-	-	TSL:1
Ryr2-205	ENSMUST00000221018.1	1572	No protein	Retained intron	-	-	TSL:5
Ryr2-206	ENSMUST00000221341.1	1335	No protein	Retained intron	-	-	TSL:2
Ryr2-210	ENSMUST00000221916.1	1316	No protein	Retained intron	-	-	TSL:5
Ryr2-209	ENSMUST00000221890.1	1196	No protein	Retained intron	-	-	TSL:5
Ryr2-212	ENSMUST00000222113.1	920	No protein	Retained intron	-	-	TSL:5
Ryr2-213	ENSMUST00000222386.1	891	No protein	Retained intron	-	-	TSL:5
Ryr2-208	ENSMUST00000221609.1	626	No protein	Retained intron	-	-	TSL:5

The strategy is based on the design of *Ryr2-201* transcript,The transcription is shown below

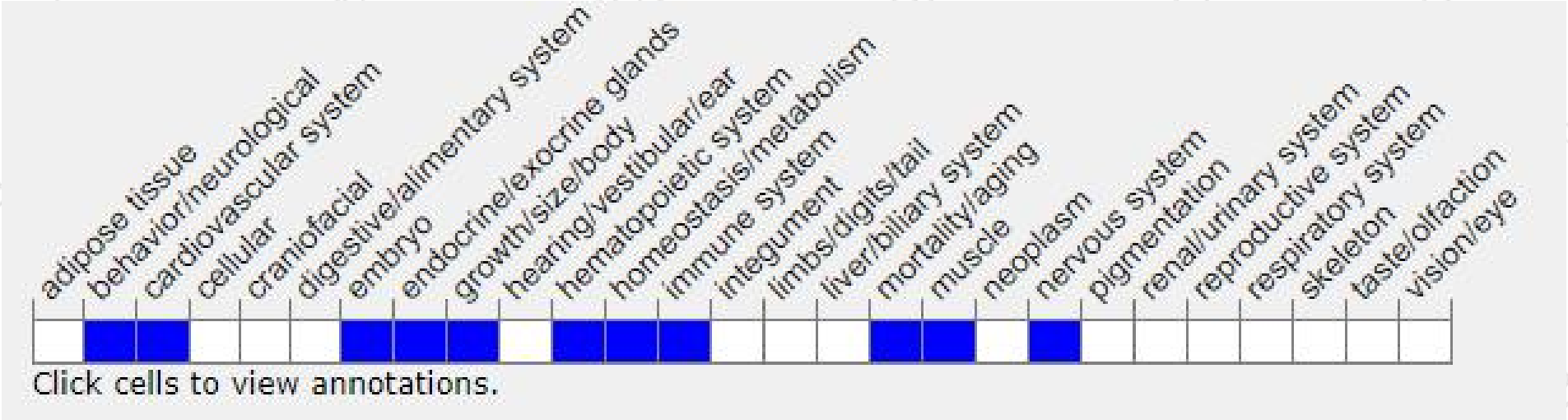


Genomic location distribution



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/marker/MGI:99685).

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

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