Ryr3 Cas9-CKO Strategy

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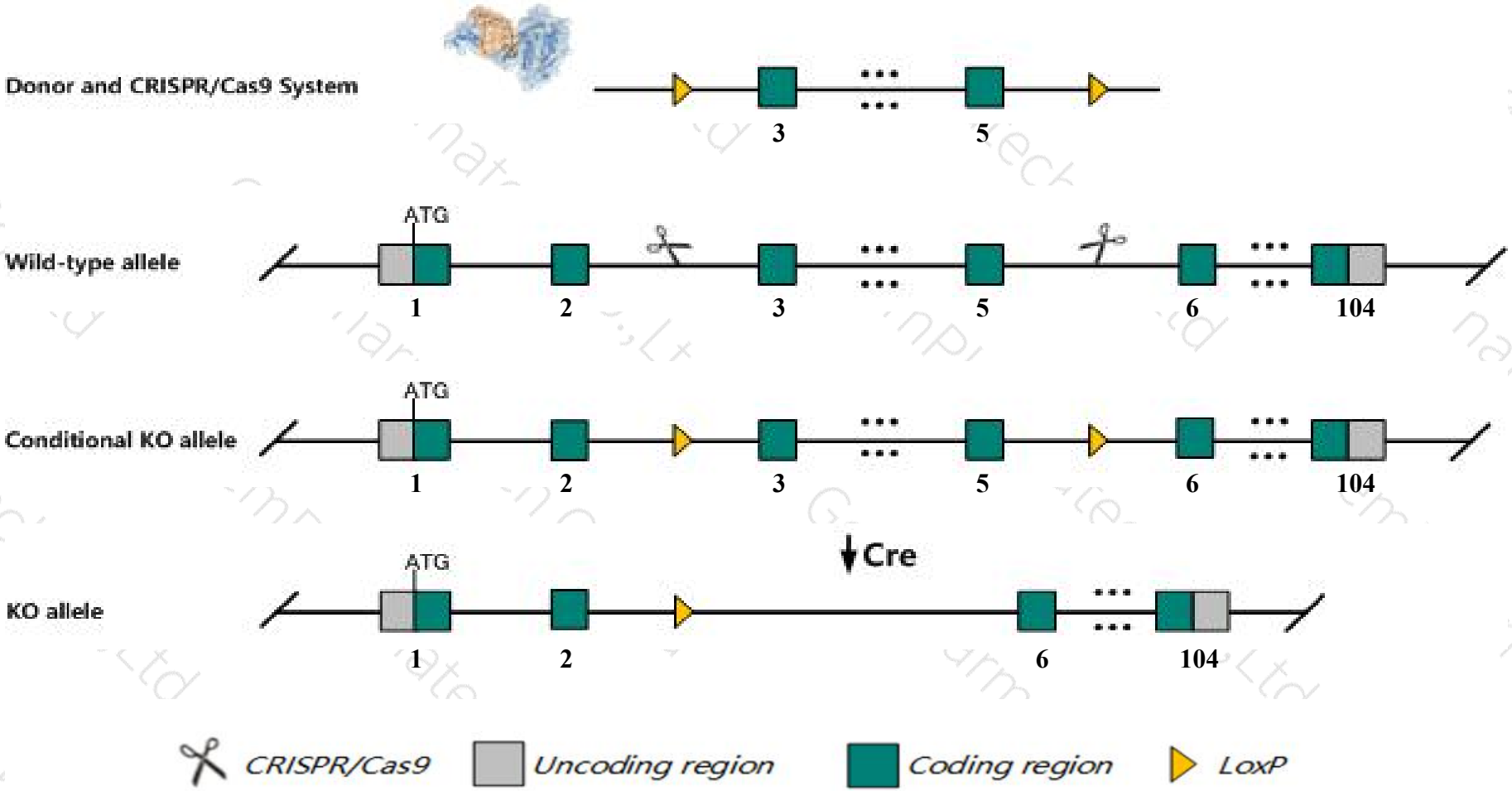
Project Overview

Project Name	***Ryr3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ryr3* gene. The schematic diagram is as follows:



Technical routes

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- The *Ryr3* gene has 12 transcripts. According to the structure of *Ryr3* gene, exon3-exon5 of *Ryr3-211* (ENSMUST00000208290.1) transcript is recommended as the knockout region.The region contains 262bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ryr3* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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, omozygotes for targeted null mutations exhibit impaired muscle contraction at an early age, changes in hippocampal synaptic plasticity, increased locomotor activity with a tendency to circle, and impaired relearning of a spatial task.

- Transcript *Ryr3*-205&206&212 may not be affected.

- The *Ryr3* gene is located on the Chr2. 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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Ryr3 ryanodine receptor 3 [*Mus musculus* (house mouse)]

Gene ID: 20192, updated on 12-Aug-2019

Summary

Official Symbol	Ryr3 provided by MGI
Official Full Name	ryanodine receptor 3 provided by MGI
Primary source	MGI:MGI:99684
See related	Ensembl:ENSMUSG00000057378
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-3; AI851294; C230090H21
Expression	Biased expression in CNS E18 (RPKM 3.5), bladder adult (RPKM 2.8) and 12 other tissues See more
Orthologs	human all

Genomic context

Location: 2; 2 E3-E4

See Ryr3 in Genome Data Viewer

Exon count: 107

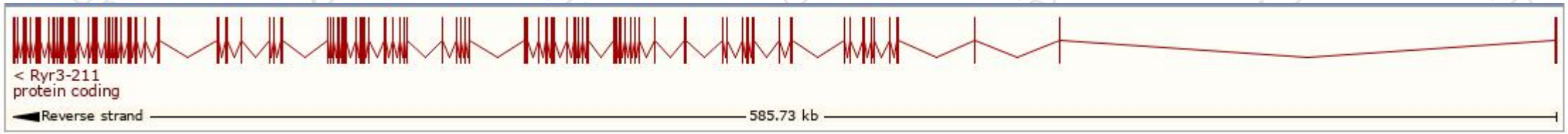
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	2	NC_000068.7 (112631354..113217405, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	2	NC_000068.6 (112471537..112870488, complement)

Transcript information（Ensembl）

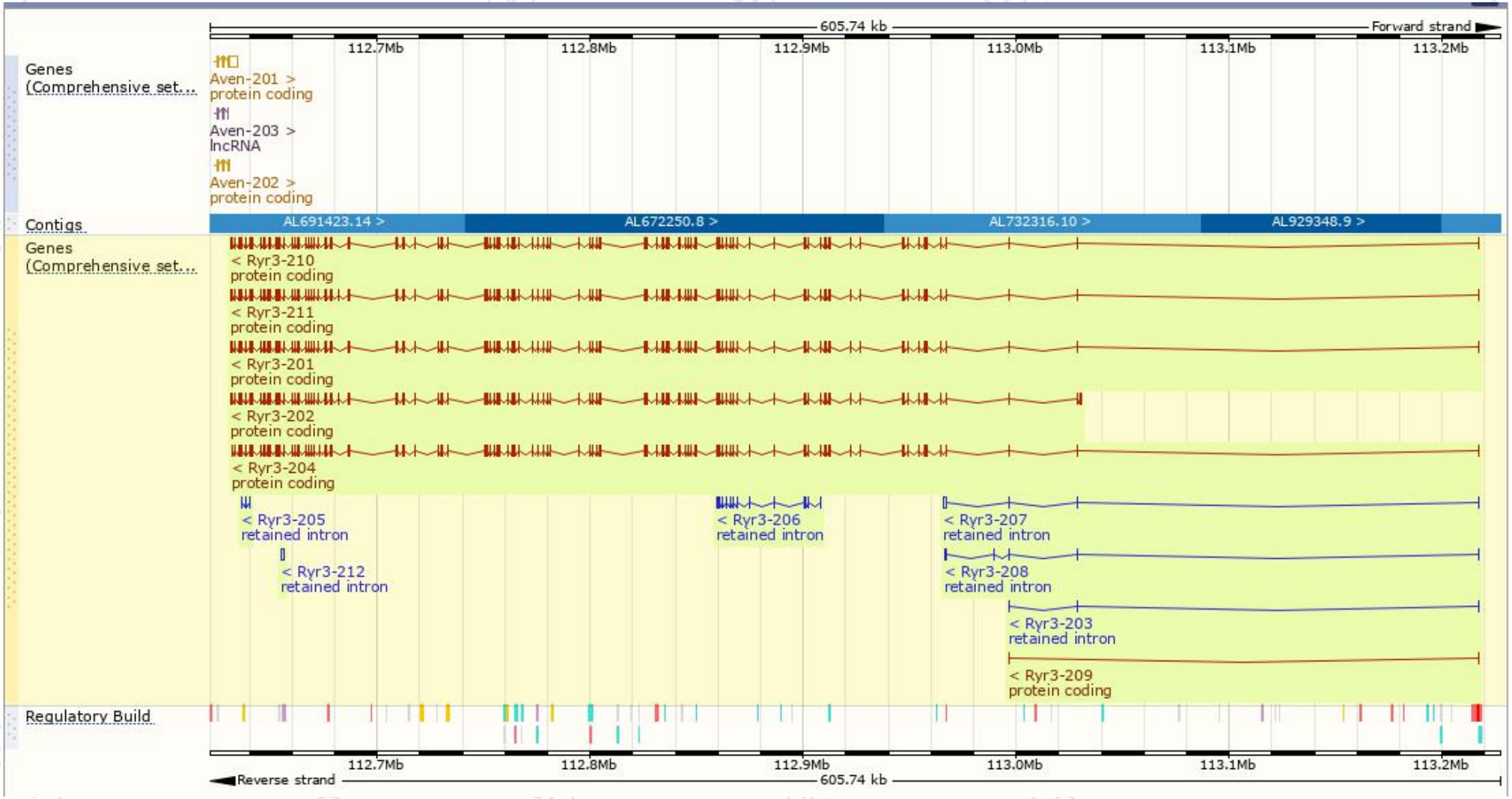
The gene has 12 transcripts,and all the transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ryr3-211	ENSMUST00000208290.1	15468	4868aa	Protein coding	CCDS50661	A0A140LJK7	TSL:5 GENCODE basic APPRIS P2
Ryr3-201	ENSMUST00000080673.12	15422	4863aa	Protein coding	-	A2AGL3	TSL:5 GENCODE basic APPRIS ALT2
Ryr3-202	ENSMUST00000091818.5	15410	4888aa	Protein coding	-	E9PW34	TSL:5 GENCODE basic
Ryr3-210	ENSMUST00000208151.1	15370	4834aa	Protein coding	-	A2AGL3	TSL:5 GENCODE basic
Ryr3-204	ENSMUST00000134358.8	14958	4858aa	Protein coding	-	A0A140LJF7	TSL:5 GENCODE basic
Ryr3-209	ENSMUST00000208135.1	137	46aa	Protein coding	-	A0A140LI87	CDS 5' and 3' incomplete TSL:5
Ryr3-206	ENSMUST00000146187.2	2239	No protein	Retained intron	-	-	TSL:1
Ryr3-207	ENSMUST00000156757.7	1730	No protein	Retained intron	-	-	TSL:1
Ryr3-212	ENSMUST00000208574.1	1325	No protein	Retained intron	-	-	TSL:NA
Ryr3-208	ENSMUST00000207603.1	1294	No protein	Retained intron	-	-	TSL:1
Ryr3-205	ENSMUST00000142537.1	612	No protein	Retained intron	-	-	TSL:3
Ryr3-203	ENSMUST00000128192.1	430	No protein	Retained intron	-	-	TSL:1

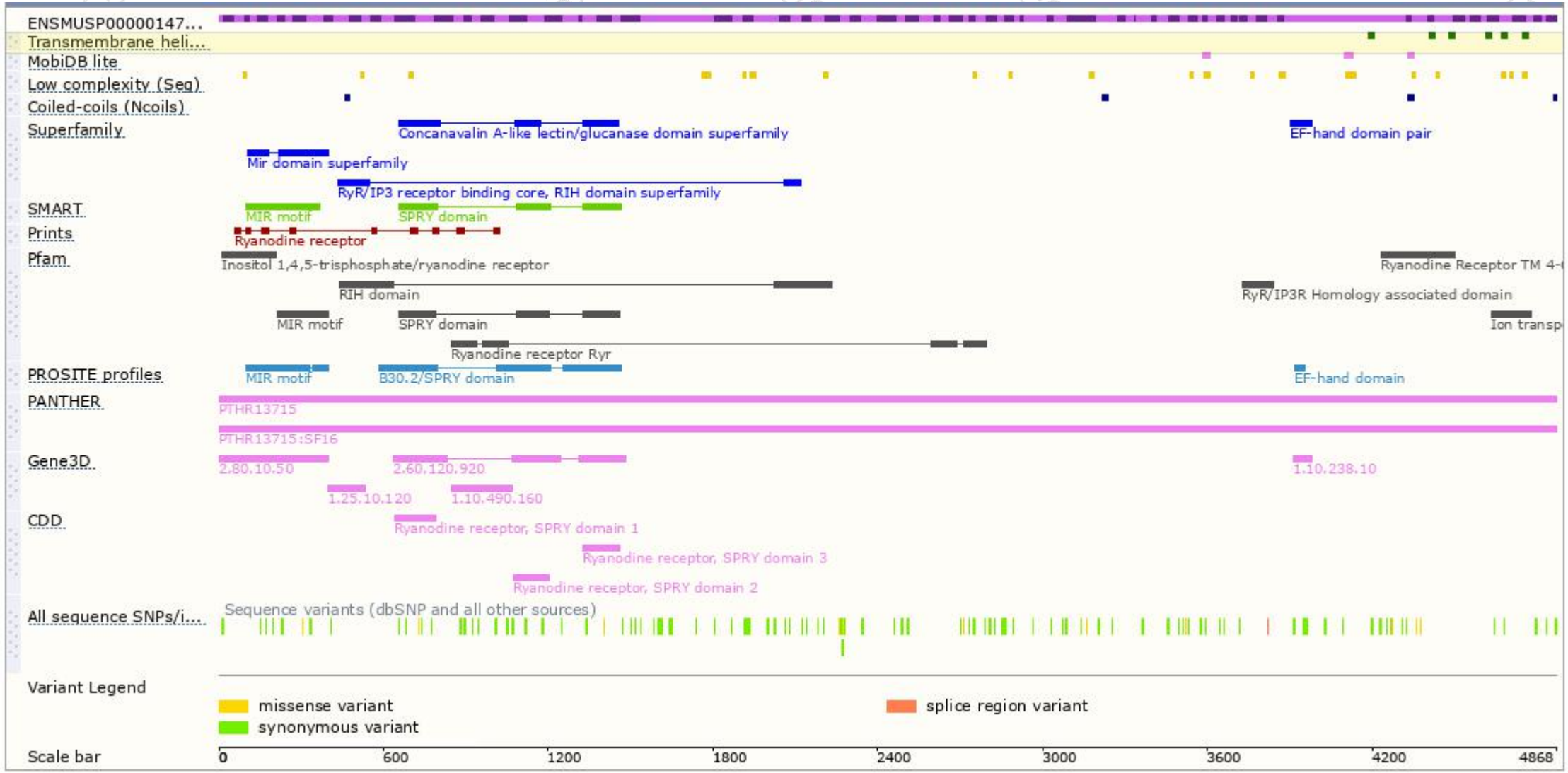
The strategy is based on the design of *Ryr3-211* transcript,The transcription is shown below



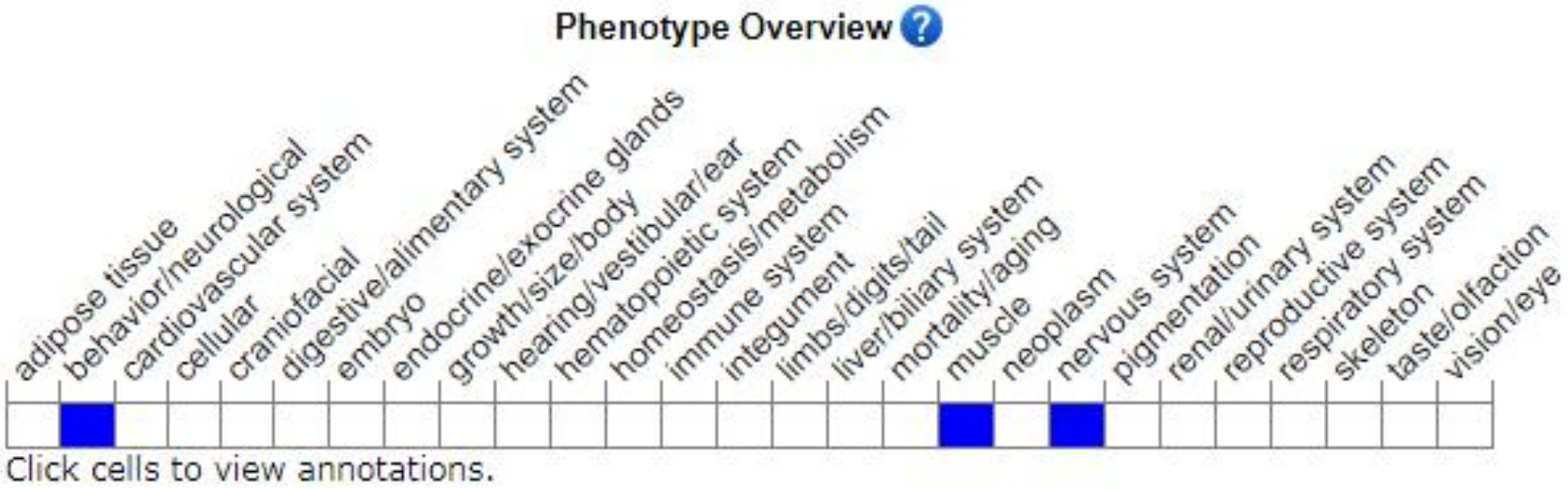
Genomic location distribution



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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