Lrrc8a Cas9-KO Strategy

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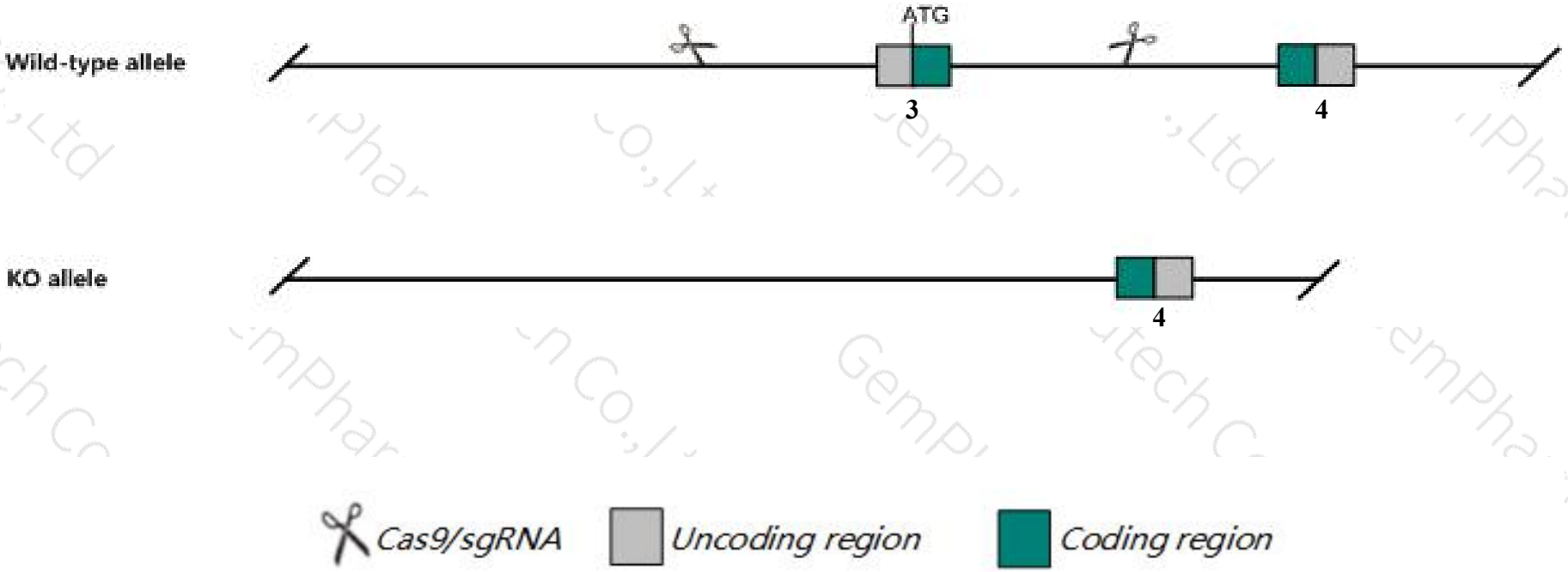
Project Overview

Project Name	***Lrrc8a***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Lrrc8a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Lrrc8a* gene has 2 transcripts. According to the structure of *Lrrc8a* gene, exon3 of *Lrrc8a-201* (ENSMUST00000095078.2) transcript is recommended as the knockout region.The region contains start codon ATG. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Lrrc8a* 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 for a knock-out allele exhibit prenatal lethality and premature death, growth retardation, sterility, multiple tissue abnormalities, a severe block in early thymic development, and impaired peripheral T cell function. B cell development is modestly impaired but B cell function is normal.

- The *Lrrc8a* 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 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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Lrrc8a leucine rich repeat containing 8A [Mus musculus (house mouse)]

Gene ID: 241296, updated on 17-Feb-2019

Summary

Official Symbol	Lrrc8a provided by MGI
Official Full Name	leucine rich repeat containing 8A provided by MGI
Primary source	MGI:MGI:2652847
See related	Ensembl:ENSMUSG00000007476
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	Lrrc8, ebo, mKIAA1437
Expression	Ubiquitous expression in lung adult (RPKM 34.6), ovary adult (RPKM 30.0) and 28 other tissues See more
Orthologs	human all

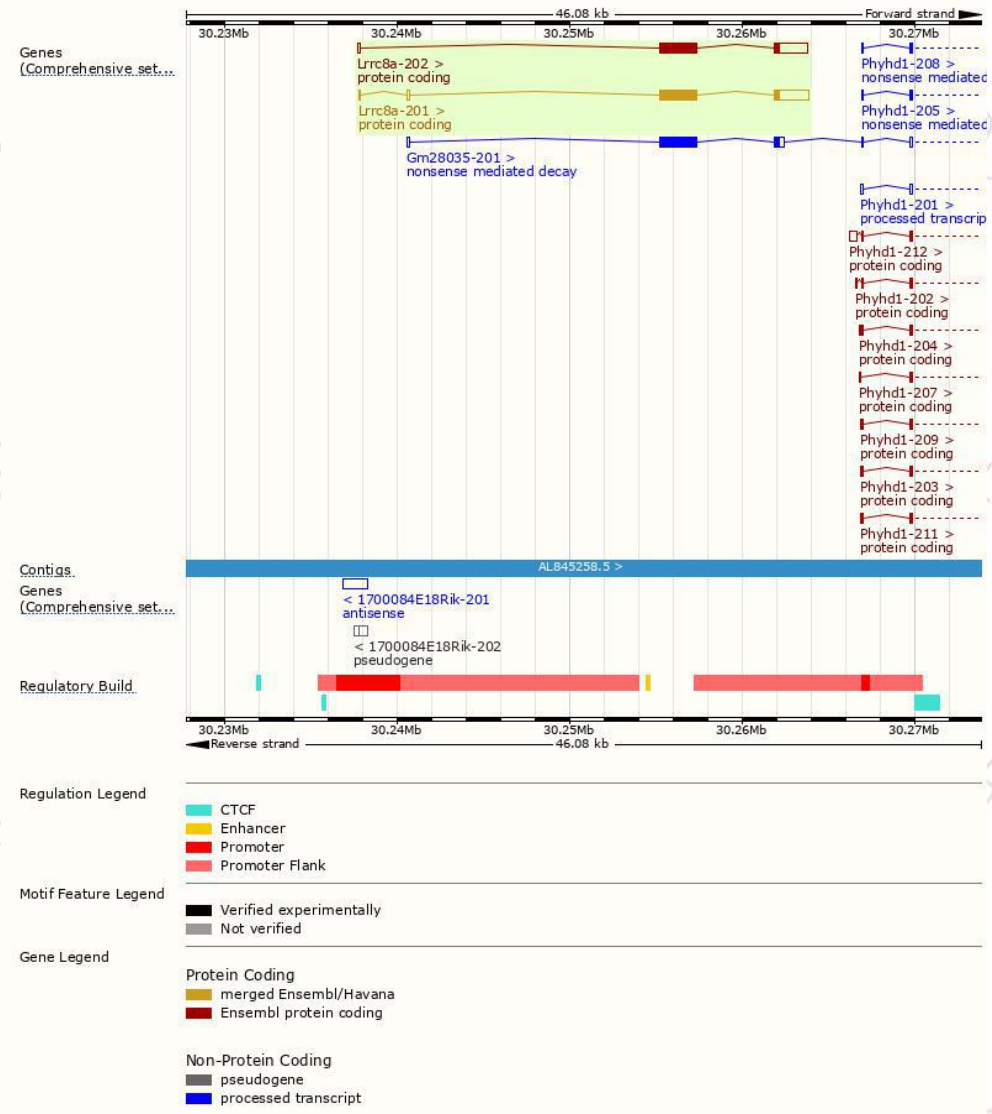
Transcript information（Ensembl）

The gene has 2 transcripts,all transcripts are shown below:

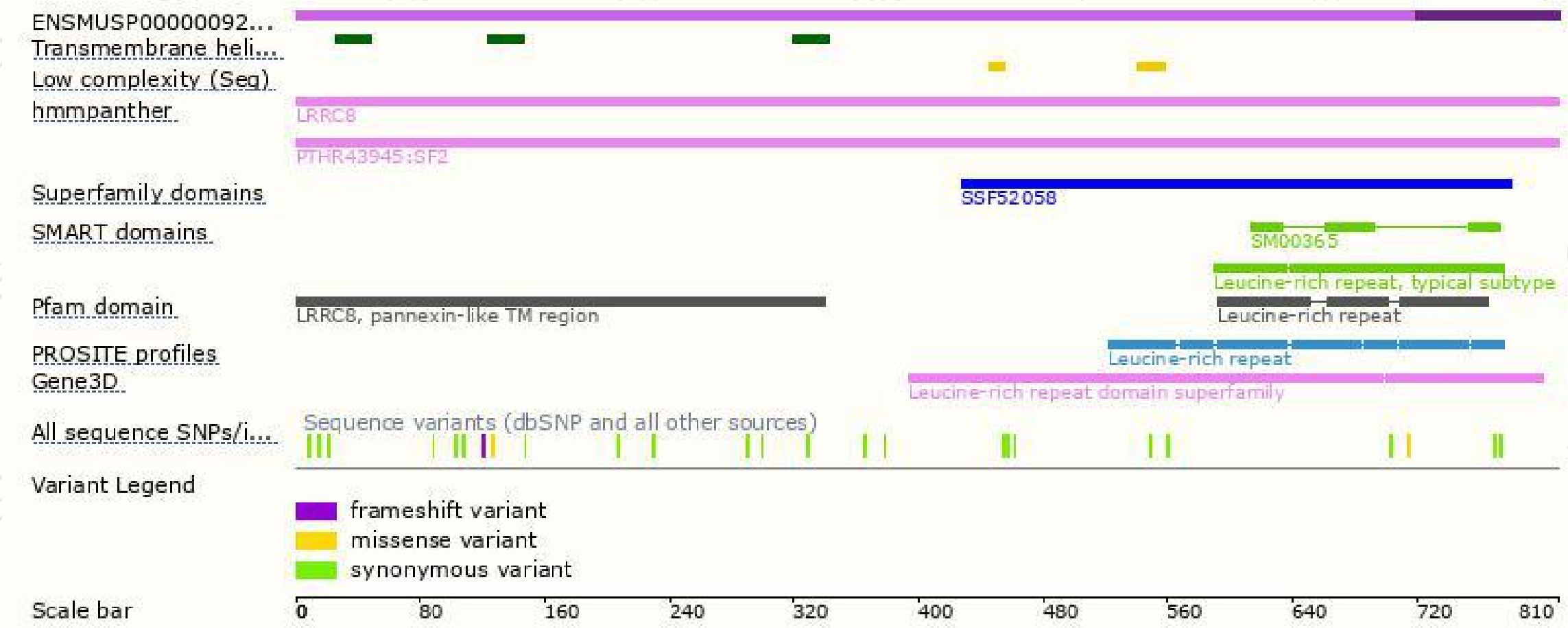
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lrrc8a-201	ENSMUST00000095078.2	4301	810aa	Protein coding	CCDS15875	Q80WG5	TSL:5 GENCODE basic APPRIS P1
Lrrc8a-202	ENSMUST00000113654.7	4194	810aa	Protein coding	CCDS15875	Q80WG5	TSL:5 GENCODE basic APPRIS P1

The strategy is based on the design of *Lrrc8a-201* transcript,The transcription is shown below

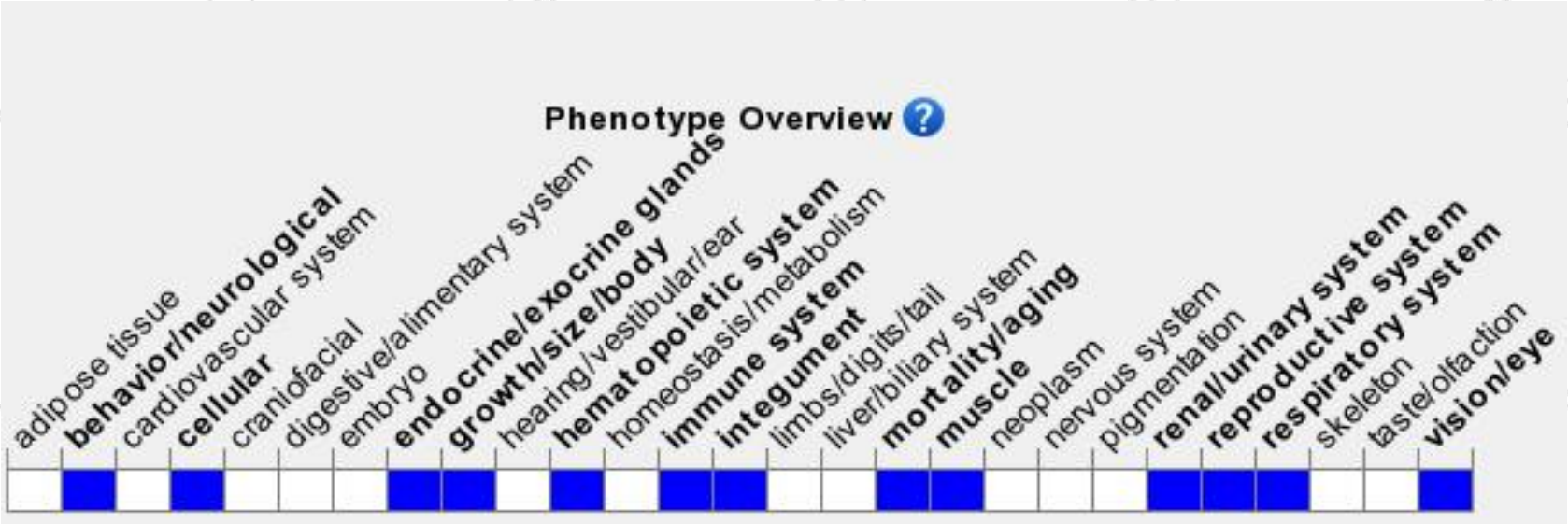
Genomic location distribution



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/).

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

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