

Lrrc8d Cas9-CKO Strategy

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Project Overview

Project Name

Lrrc8d

Project type

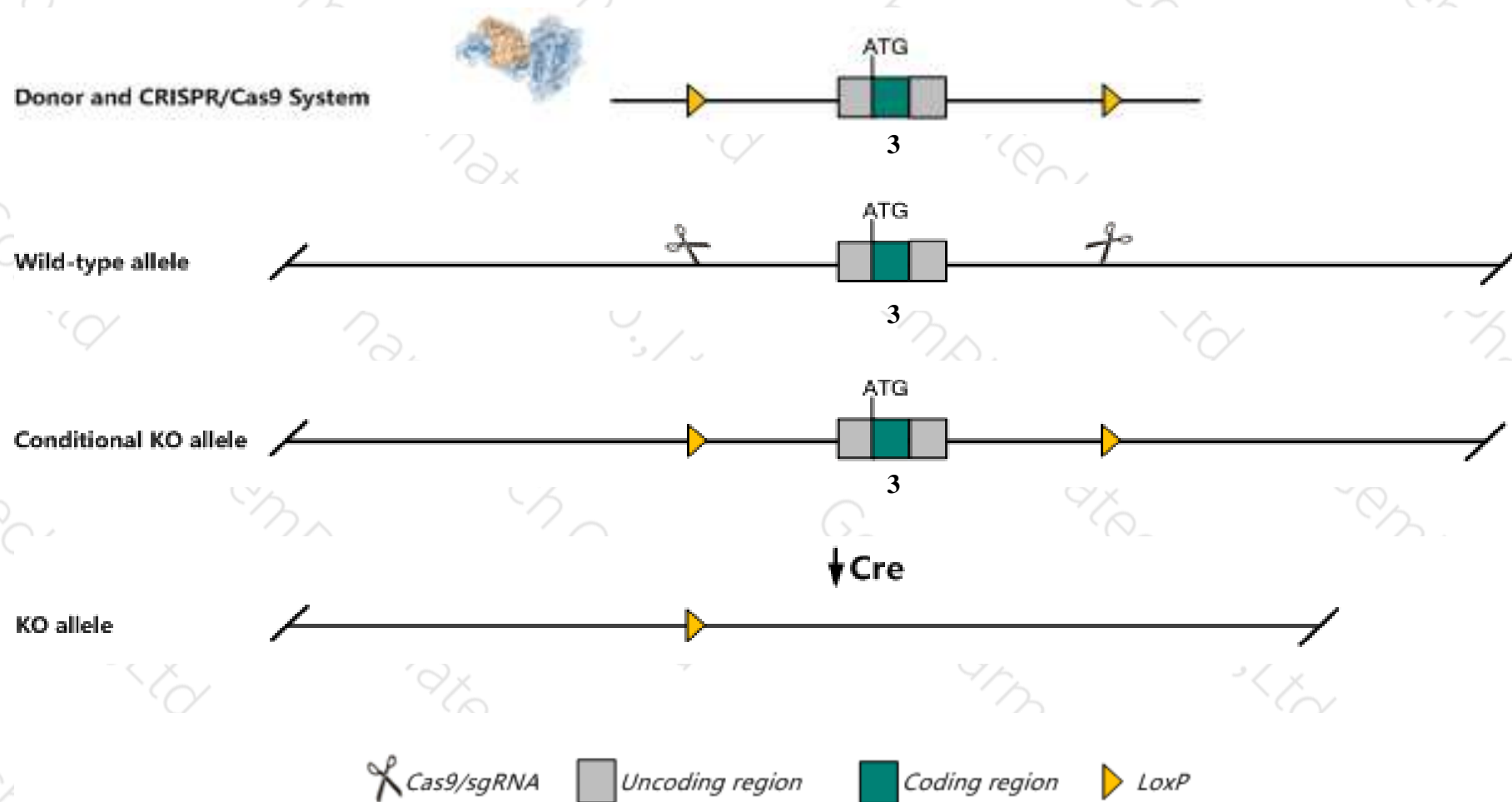
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Lrrc8d* gene. The schematic diagram is as follows:



Technical routes

- The *Lrrc8d* gene has 10 transcripts. According to the structure of *Lrrc8d* gene, exon3 of *Lrrc8d*-202(ENSMUST00000120847.7) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Lrrc8d* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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 was 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.

- The KO region contains functional region of the *Lrrc8dos* gene. Knockout the region may affect the function of *Lrrc8dos* gene.
- The *Lrrc8d* gene is located on the Chr5. 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)

Lrrc8d leucine rich repeat containing 8D [Mus musculus (house mouse)]

Gene ID: 231549, updated on 13-Mar-2020

Summary



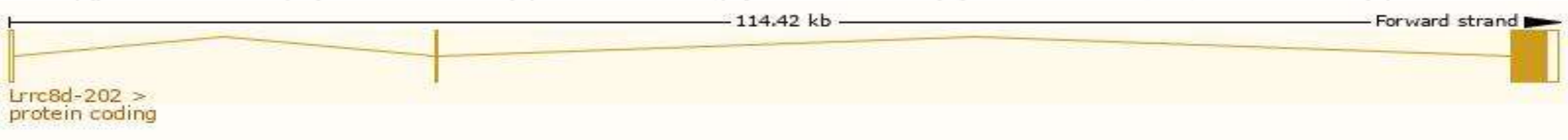
Official Symbol	Lrrc8d provided by MGI
Official Full Name	leucine rich repeat containing 8D provided by MGI
Primary source	MGI:MGI:1922368
See related	Ensembl:ENSMUSG00000046079
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2810473G09Rik, 4930525N13Rik, A930019F03, Lrrc5
Expression	Ubiquitous expression in kidney adult (RPKM 6.4), large intestine adult (RPKM 3.9) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

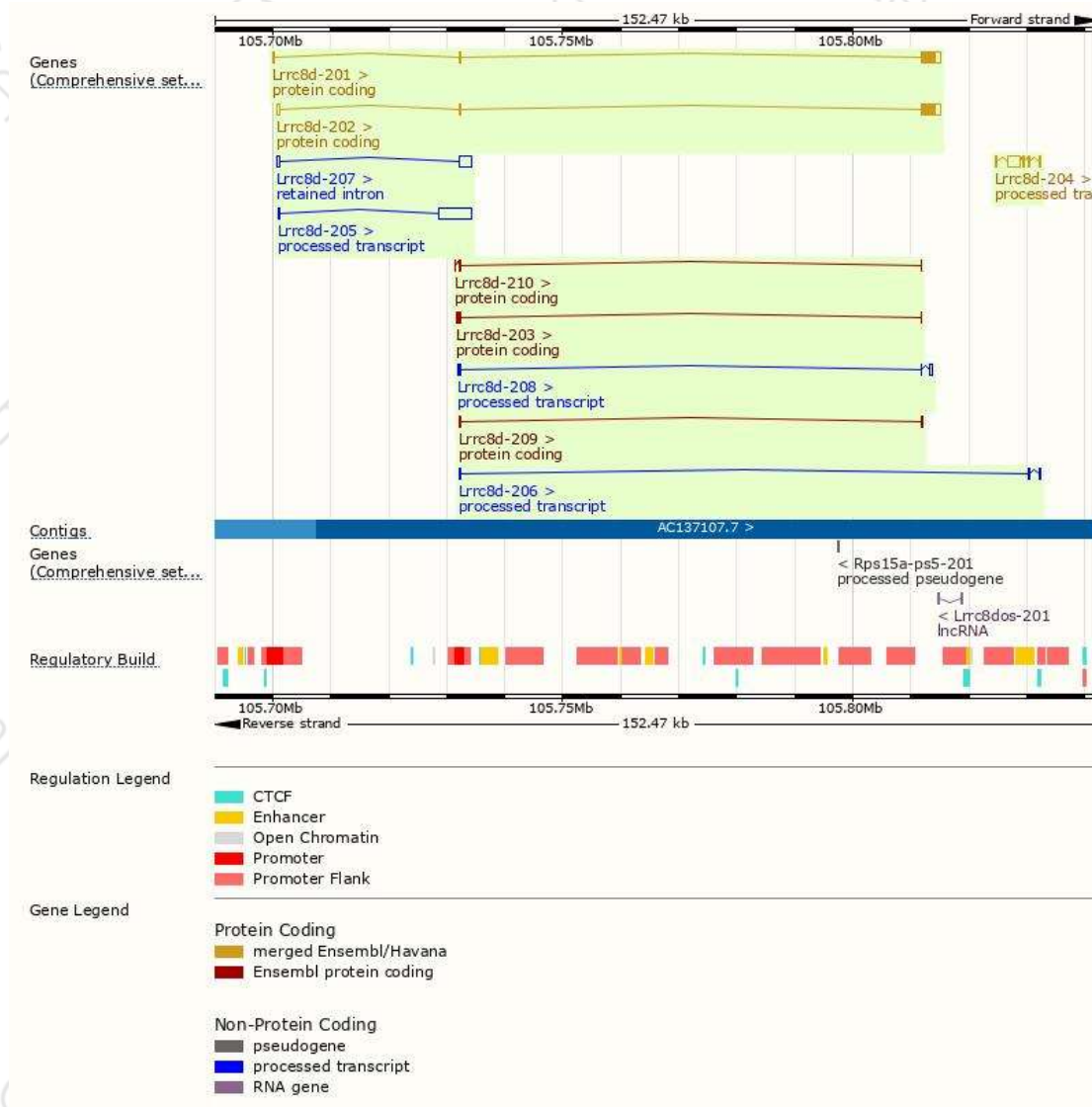
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lrrc8d-202	ENSMUST00000120847.7	3929	859aa	Protein coding	CCDS19494	Q8BGR2	TSL:1 GENCODE basic APPRIS P1
Lrrc8d-201	ENSMUST00000060531.15	3923	859aa	Protein coding	CCDS19494	Q8BGR2	TSL:1 GENCODE basic APPRIS P1
Lrrc8d-209	ENSMUST00000154807.1	593	93aa	Protein coding	-	D3YV62	CDS 3' incomplete TSL:1
Lrrc8d-203	ENSMUST00000127686.7	498	41aa	Protein coding	-	D3Z6C1	CDS 3' incomplete TSL:3
Lrrc8d-210	ENSMUST00000156630.7	366	37aa	Protein coding	-	D3Z5Q6	CDS 3' incomplete TSL:5
Lrrc8d-205	ENSMUST00000135776.1	5736	No protein	Processed transcript	-	-	TSL:1
Lrrc8d-204	ENSMUST00000134605.1	2979	No protein	Processed transcript	-	-	TSL:1
Lrrc8d-208	ENSMUST00000149831.7	500	No protein	Processed transcript	-	-	TSL:5
Lrrc8d-206	ENSMUST00000140081.7	492	No protein	Processed transcript	-	-	TSL:2
Lrrc8d-207	ENSMUST00000140608.1	2287	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Lrrc8d-202* transcript,the transcription is shown below:



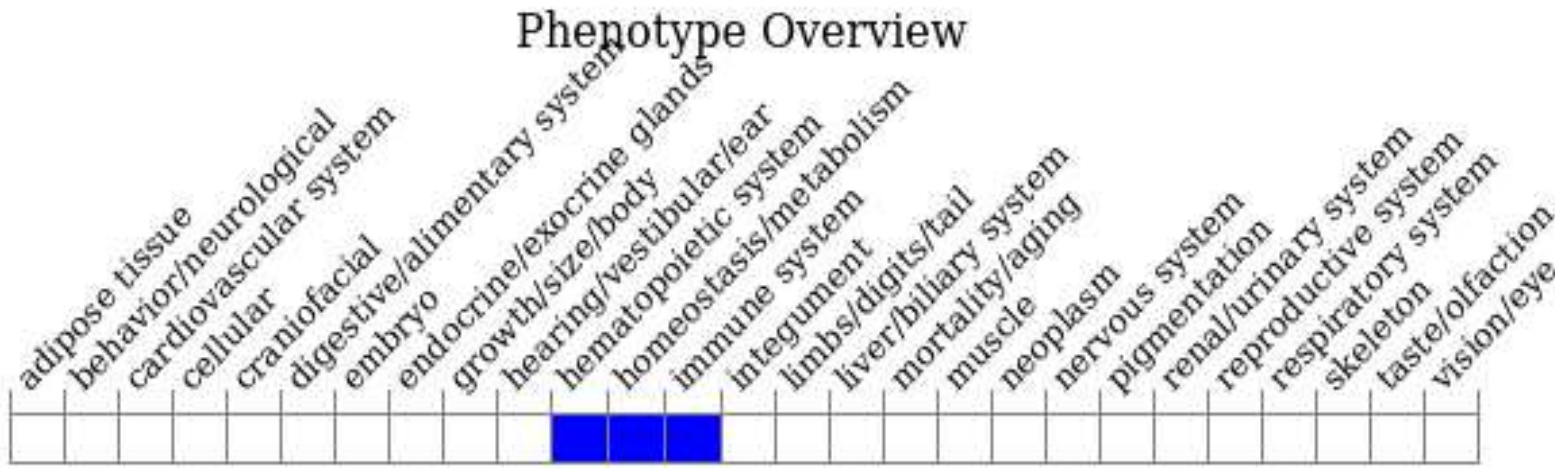
Genomic location distribution



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/>).

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

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