Lrp4 Cas9-CKO Strategy

Designer: Miaomiao Cui

Reviewer: Shilei Zhu

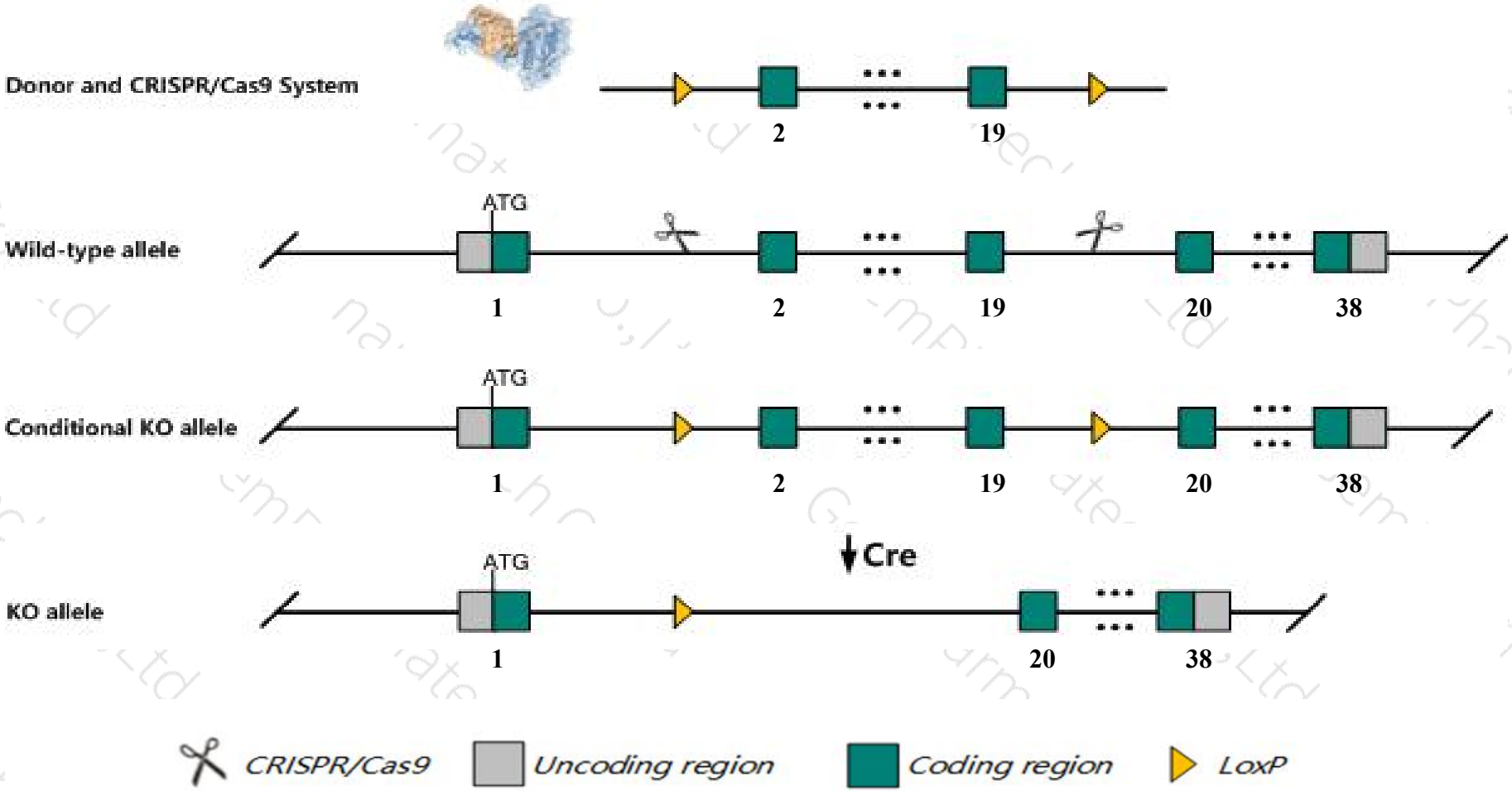
Design Date: 2020-8-13

Project Overview

Project Name	***Lrp4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Lrp4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Lrp4* gene has 4 transcripts. According to the structure of *Lrp4* gene, exon2-exon19 of *Lrp4-201*(ENSMUST00000028689.3) transcript is recommended as the knockout region.The region contains 2560bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Lrp4* 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,homozygous mutations of this gene cause polysyndactyly. Additional phenotypes may include growth retardation, abnormal incisor development, kidney agenesis, and neonatal lethality associated with respiratory failure.

➢ The *Lrp4* 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）

集萃药康 GemPharmatech

Lrp4 low density lipoprotein receptor-related protein 4 [Mus musculus (house mouse)]

Gene ID: 228357, updated on 20-Mar-2020

Summary

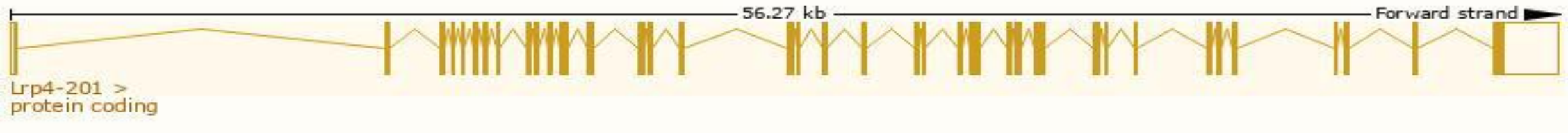
Official Symbol	Lrp4 provided by MGI
Official Full Name	low density lipoprotein receptor-related protein 4 provided by MGI
Primary source	MGI:MGI:2442252
See related	Ensembl:ENSMUSG00000027253
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	6430526J12Rik, D230026E03, Megf7, mdig
Expression	Broad expression in lung adult (RPKM 19.7), genital fat pad adult (RPKM 6.0) and 20 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

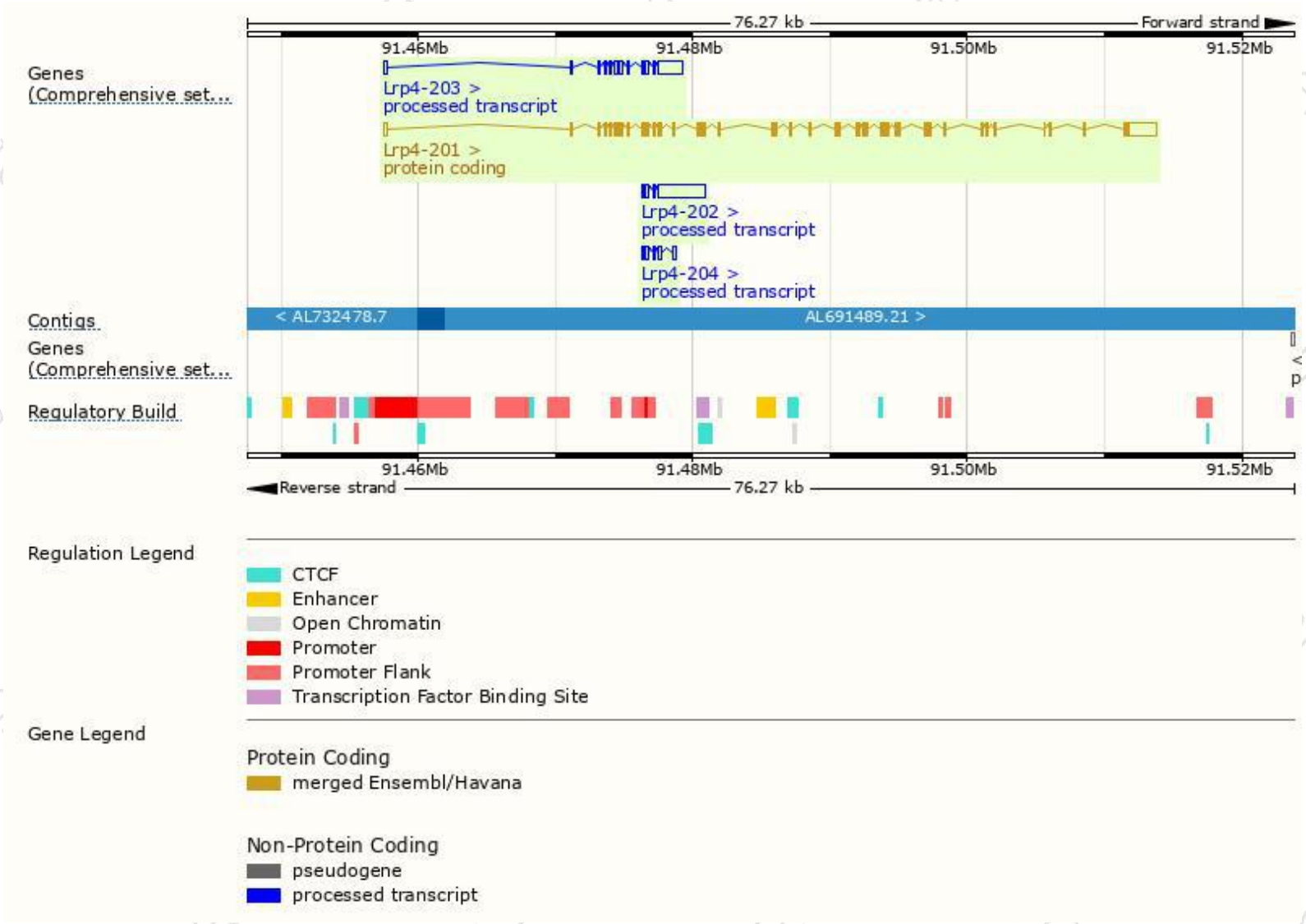
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lrp4-201	ENSMUST00000028689.3	7926	1905aa	Protein coding	CCDS16432	Q8VI56	TSL:1 GENCODE basic APPRIS P1
Lrp4-202	ENSMUST00000123897.7	3748	No protein	Processed transcript	-	-	TSL:2
Lrp4-203	ENSMUST00000143874.7	3266	No protein	Processed transcript	-	-	TSL:1
Lrp4-204	ENSMUST00000151907.1	898	No protein	Processed transcript	-	-	TSL:1

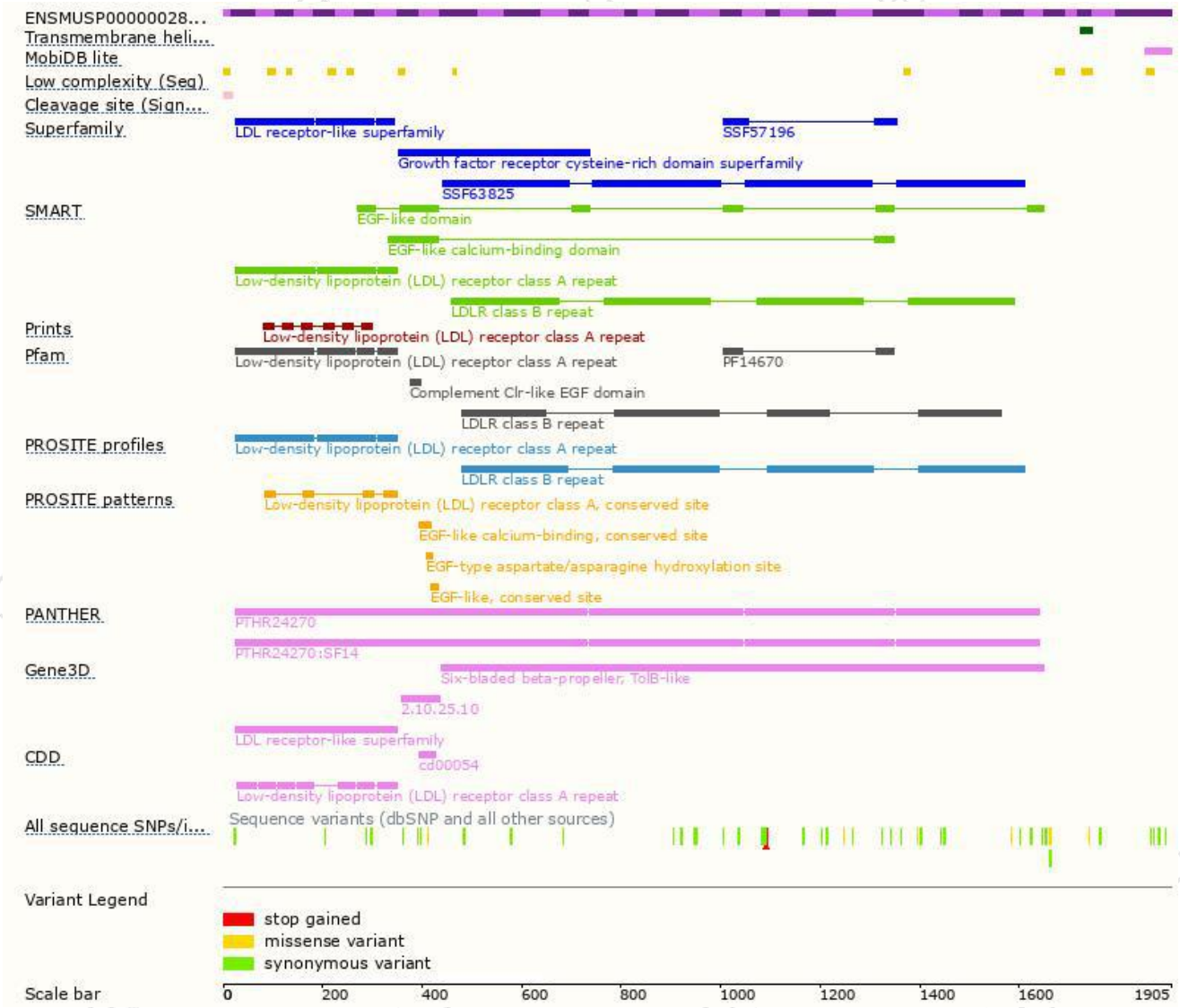
The strategy is based on the design of *Lrp4-201* transcript,the transcription is shown below:



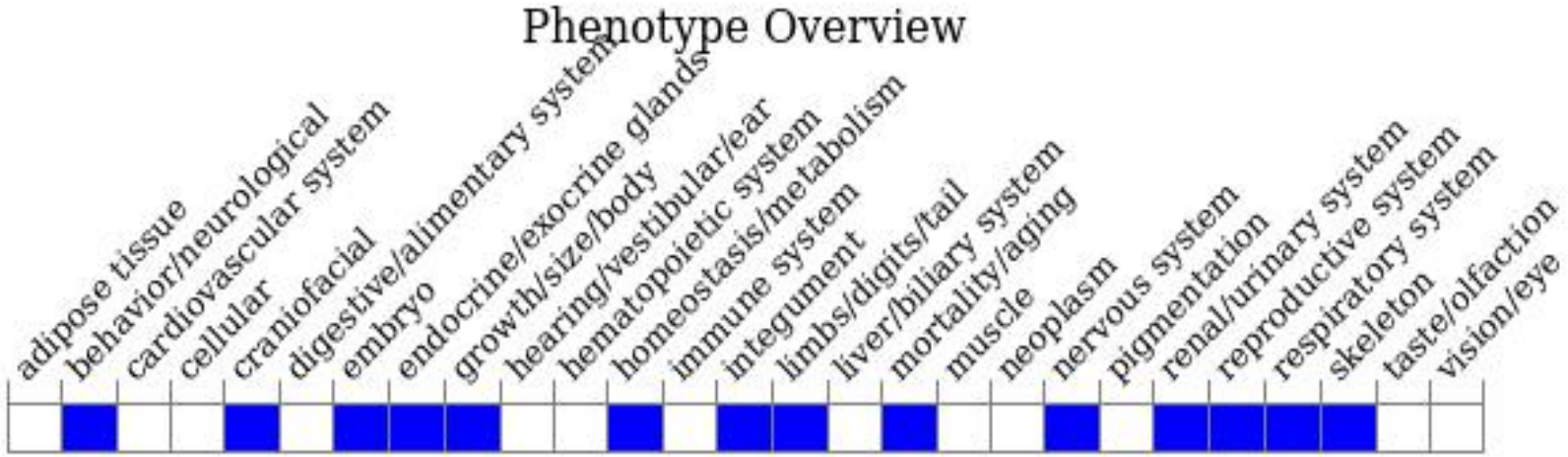
Genomic location distribution



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/).

According to the existing MGI data,homozygous mutations of this gene cause polysyndactyly. Additional phenotypes may include growth retardation, abnormal incisor development, kidney agenesis, and neonatal lethality associated with respiratory failure.

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd