Lrp2 Cas9-CKO Strategy

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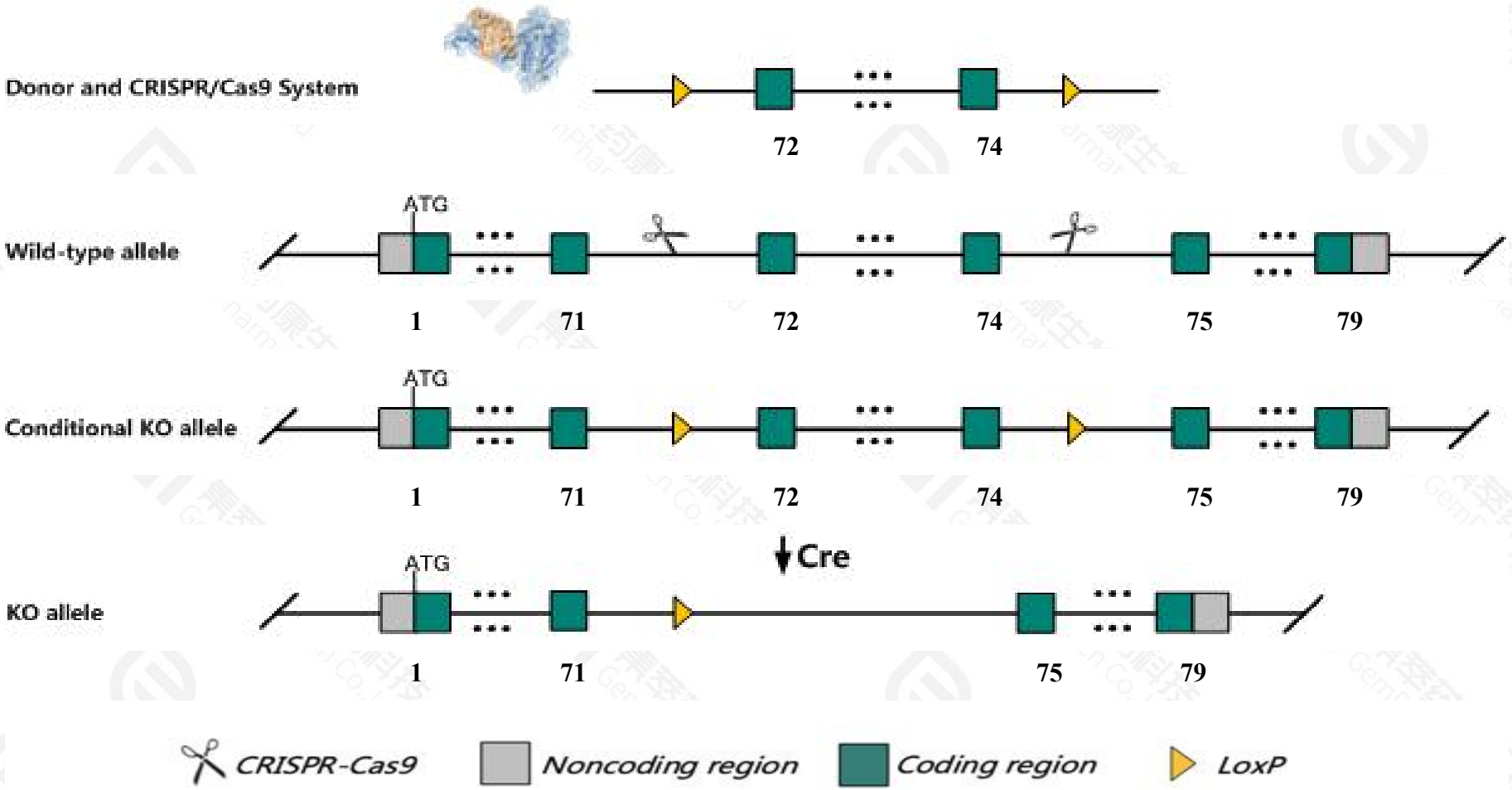
Design Date: 2022-6-10

Project Overview

Project Name	***Lrp2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR-Cas9 technology to edit the *Lrp2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Lrp2* gene has 4 transcripts. According to the structure of *Lrp2* gene, exon72-exon74 of *Lrp2-201*(ENSMUST00000080953.12) transcript is recommended as the knockout region.The region contains 277bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR-Cas9 technology to modify *Lrp2* 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,homozygotes for a targeted null mutation exhibit lung and kidney epithelial defects, impaired B12 uptake, reduced proliferation of the neuroepithelium resulting in lack of olfactory bulbs, forebrain fusions, ventricular defects, and perinatal lethality.

➢ The N-terminal of *Lrp2* gene will remain amino acids, it may remain the partial function of *Lrp2* gene.

➢ Transcript *Lrp2*-202 & 203 may not be affected.

➢ The *Lrp2* 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.

Published CKO model

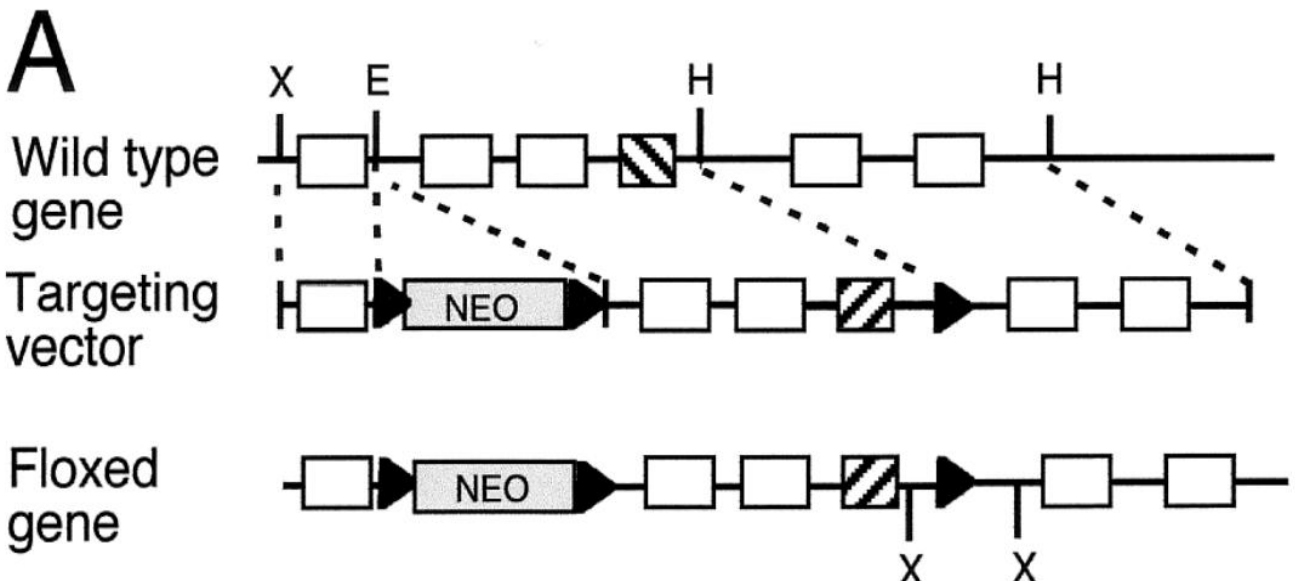


Figure 1. Gene targeting of the murine megalin gene locus. A) The exon (open box) and intron structure (solid line) of part of the murine megalin gene is depicted. The striped box represents the exon encoding the membrane anchor of the receptor. The targeting vector was constructed by inserting the *pGKneo* selection cassette (NEO) flanked by two *lox P* sites (filled triangles) into an *EcoRI* restriction site (E) and third *lox P* site into a *HindIII* restriction site (H) of the wild-type (wt) gene. Following homologous recombination, targeting of the megalin gene (floxed gene) was detected by Southern blot analysis of an *XbaI* (X) fragment harboring the third *lox P* site. **B)** Genomic DNA (20 μg) of either wild-type (lanes 2 and 4), heterozygous (lane 1), or homozygous mice for the *lox P*-modified megalin gene (floxed, lane 3) was digested with *XbaI* and analyzed by Southern blotting. The 1.6-kb *XbaI* fragment of the wild-type and the 1.7-kb *XbaI* fragment of the floxed megalin gene locus are indicated.

MATERIALS AND METHODS

Generation of mouse lines megalin$^{lox/lox}$, apoE^{Cre}, and (megalin$^{lox/lox}$; apoE^{Cre})

A targeting vector was generated to introduce *lox P* recombination sites into the megalin gene locus. The structure of the vector is depicted in Figure 1A. In brief, a 1-kb *XbaI/EcoRI*, a 3.2-kb *EcoRI/HindIII*, and a 4-kb *HindIII* fragment of the murine megalin gene were inserted into vector *pFlox* that contains the neomycin phosphotransferase gene driven by the phosphoglycerate kinase promoter and three *lox P* sites (kindly provided by J. Herz, University of Texas Southwestern Medical Center). Electroporation of the vector into murine embryonic stem (ES) cells and derivation of germ line chimeras were performed according to standard procedures. Mice homozygous for the *lox P*-modified megalin gene (megalin$^{lox/lox}$) were viable

[1] Leheste JR, et al, Hypocalcemia and osteopathy in mice with kidney-specific megalin gene defect. FASEB J 17: 247–249, 2003

Gene information（NCBI）

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Lrp2 low density lipoprotein receptor-related protein 2 [Mus musculus (house mouse)]

Gene ID: 14725, updated on 13-Mar-2020

Summary

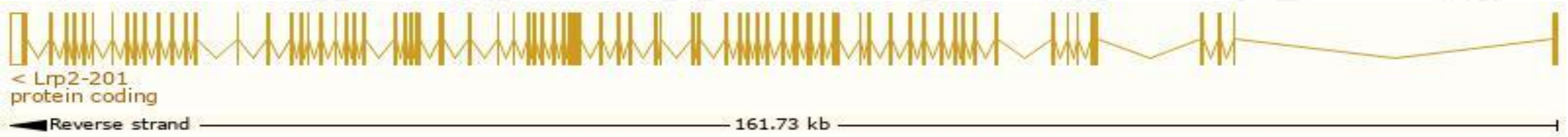
Official Symbol	Lrp2 provided by MGI
Official Full Name	low density lipoprotein receptor-related protein 2 provided byMGI
Primary source	MGI:MGI:95794
See related	Ensembl:ENSMUSG00000027070
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI315343, AW536255, D230004K18Rik, Gp330, Megalin, b2b1625.2Clo
Expression	Biased expression in kidney adult (RPKM 131.9) and placenta adult (RPKM 33.9)See more
Orthologs	human all

Transcript information（Ensembl）

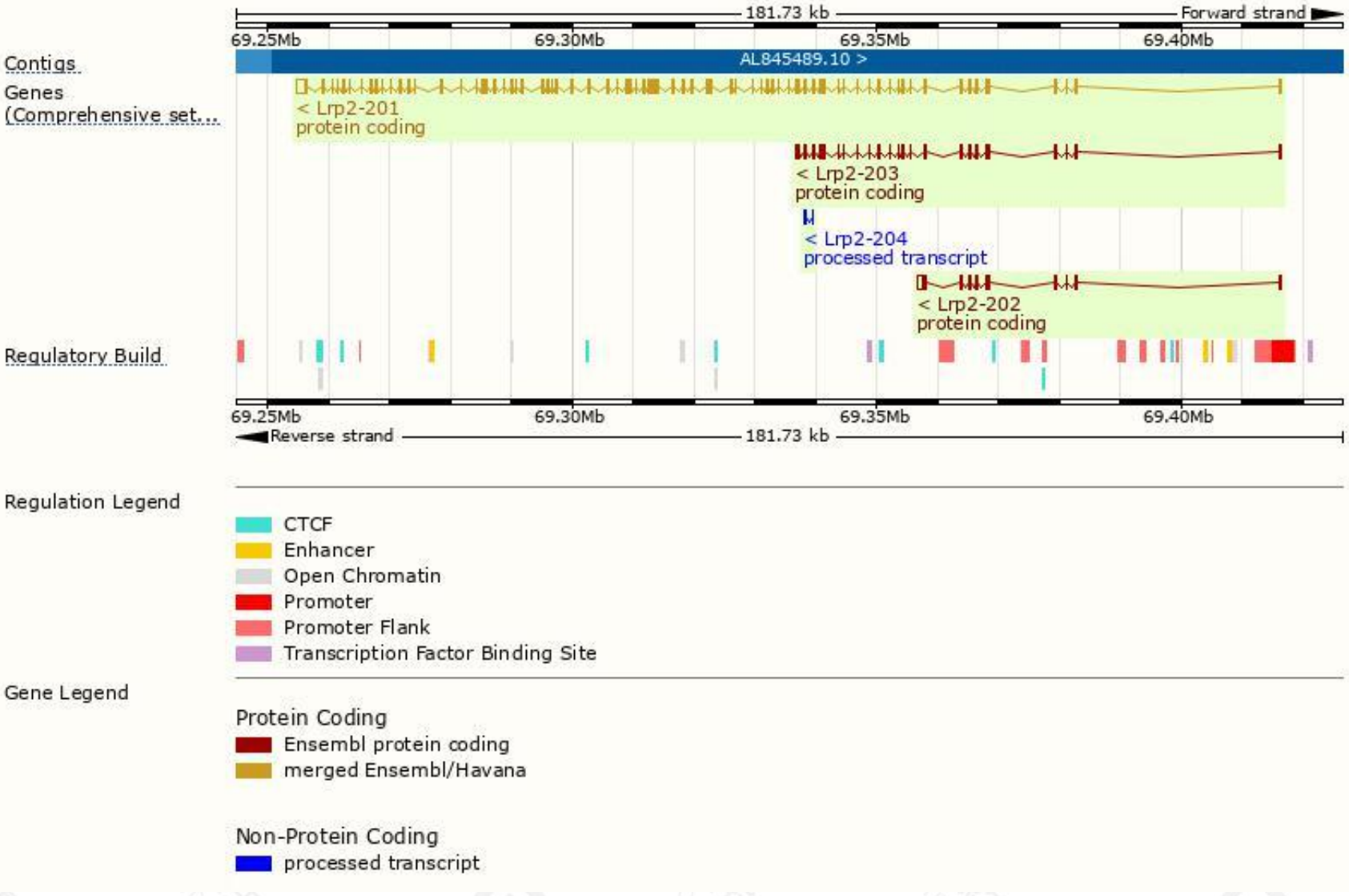
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lrp2-201	ENSMUST00000080953.11	15460	4660aa	Protein coding	CCDS38135	A2ARV4	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Lrp2-203	ENSMUST00000100051.8	4359	1363aa	Protein coding	-	A2ARV5	TSL:1 GENCODE basic
Lrp2-202	ENSMUST00000092551.4	2228	426aa	Protein coding	-	Q3V346	TSL:1 GENCODE basic
Lrp2-204	ENSMUST00000128742.1	392	No protein	Processed transcript	-	-	TSL:5

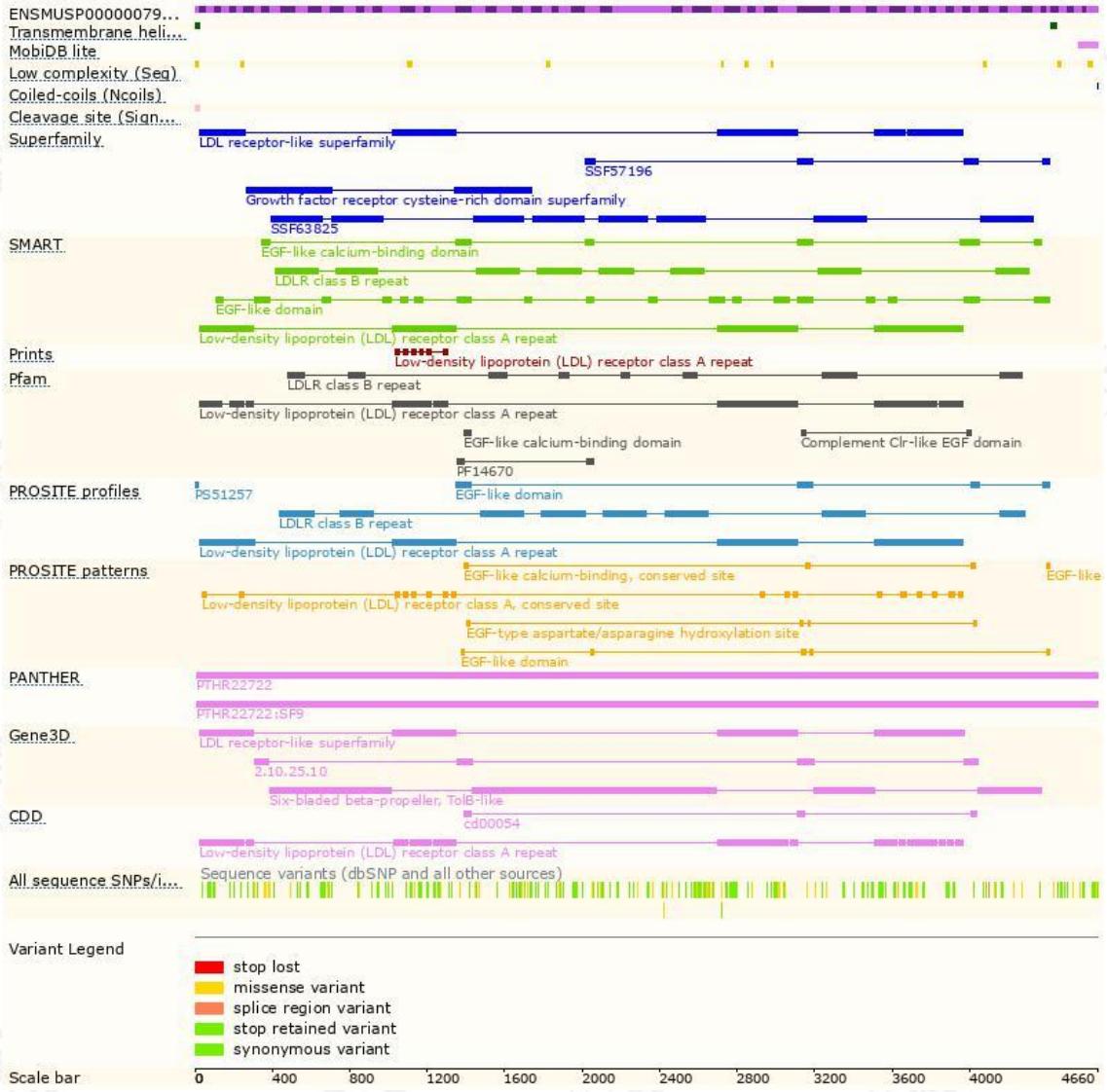
The strategy is based on the design of *Lrp2-201* transcript,the transcription is shown below:



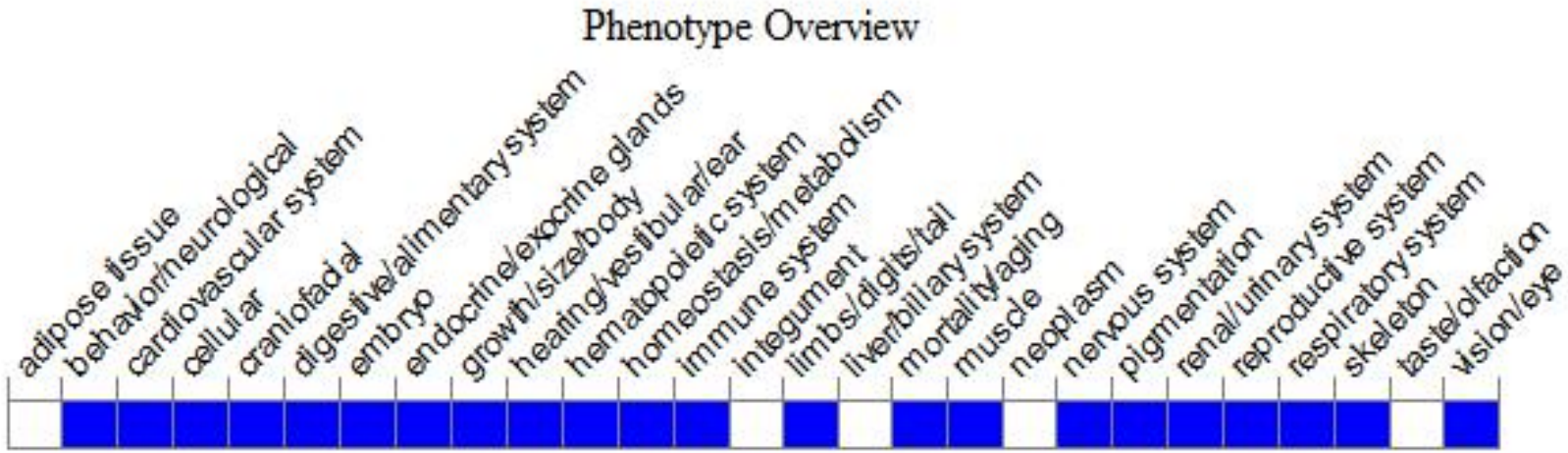
Genomic location distribution



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,homozygotes for a targeted null mutation exhibit lung and kidney epithelial defects, impaired B12 uptake, reduced proliferation of the neuroepithelium resulting in lack of olfactory bulbs, forebrain fusions, ventricular defects, and perinatal lethality.

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