Lrat-P2A-iCre Cas9-KI Strategy

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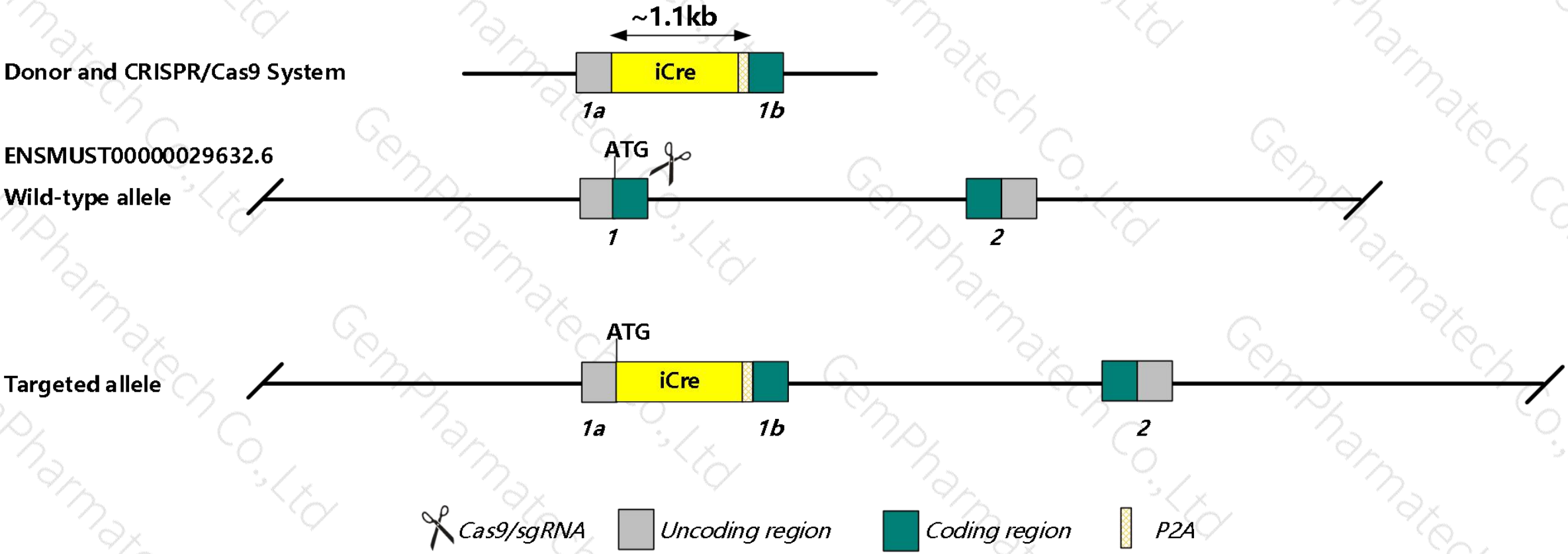
Design Date:	**2019-8-14**
Reviewer	**Xiaojing Li**

Project Overview

Project Name	***Lrat-P2A-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Conditional Knockout strategy

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



Technical routes

- The *Lrat* gene has 1 transcripts.According to the structure of *Lrat* gene, *Lrat-201*(ENSMUST00000029632.6) is selected for presentation of the recommended strategy.

- *Lrat-201* gene has 2 exons, with the ATG start codon in exon1 and TAG stop codon in exon2.

- We make *Lrat-P2A-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near start coding(ATG) of *Lrat* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in Cre-P2A after start coding(ATG) of *Lrat* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data， Mice homozygous for disruptions in this gene exhibit retinol homeostasis abnormalities and are more susceptible to vitamin A deficiency or display impaired vision associated with abnormal retinol metabolism. Males have testicular hypoplasia/atrophy and reduced mature sperm counts.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- Insertion of iCre may affect the regulation of the 5' end of the Lrat gene.
- The *Lrat* gene is located on the Chr3. If the knockin 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

集萃药康 GemPharmatech

Lrat lecithin-retinol acyltransferase (phosphatidylcholine-retinol-O-acyltransferase) [*Mus musculus* (house mouse)]

Gene ID: 79235, updated on 18-Jun-2019

Summary

Official Symbol	Lrat provided by MGI
Official Full Name	lecithin-retinol acyltransferase (phosphatidylcholine-retinol-O-acyltransferase) provided by MGI
Primary source	MGI:MGI:1891259
See related	Ensembl:ENSMUSG00000028003
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	AI449251; 1300010A18Rik
Expression	Biased expression in lung adult (RPKM 4.4), ovary adult (RPKM 2.9) and 13 other tissues See more
Orthologs	human all

Genomic context

Location: 3; 3 E3

See Lrat in Genome Data Viewer

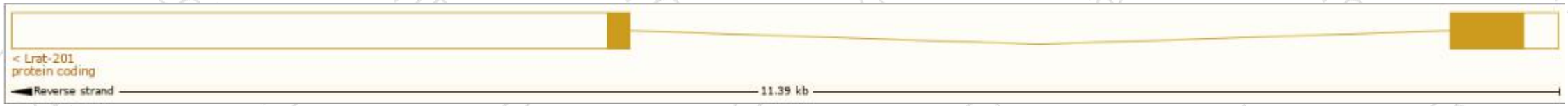
Exon count: 2

Transcript information （Ensembl）

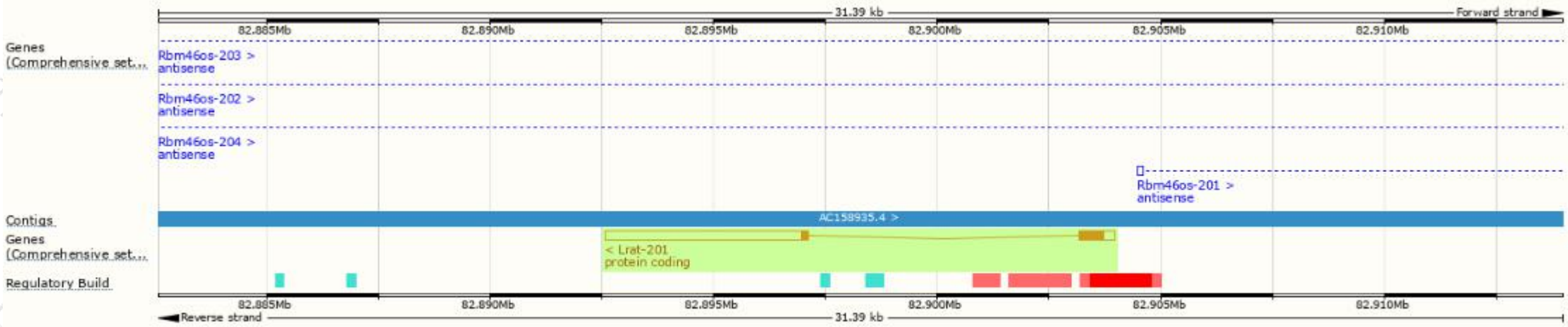
The gene has 1 transcripts, and all transcripts are shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags		
Lrat-201	ENSMUST00000029632.6	5351	231aa	Protein coding	CCDS17430	B2RUR5 Q9JI60	TSL:1	GENCODE basic	APPRIS P1

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

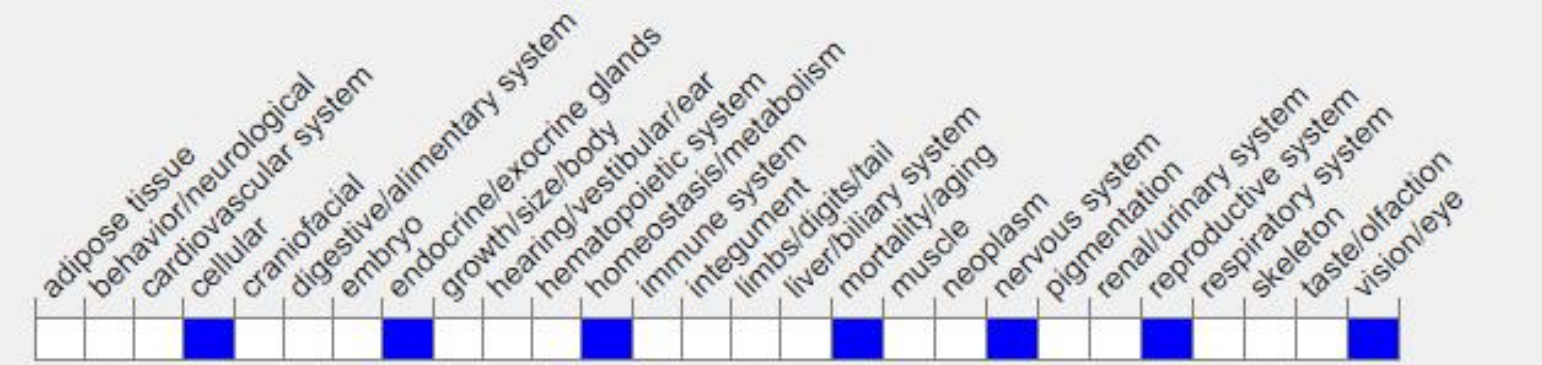


Genomic location distribution



Protein domain

Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI (http://www.informatics.jax.org/marker/MGI:1891259) .

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

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