Lctl Cas9-CKO Strategy

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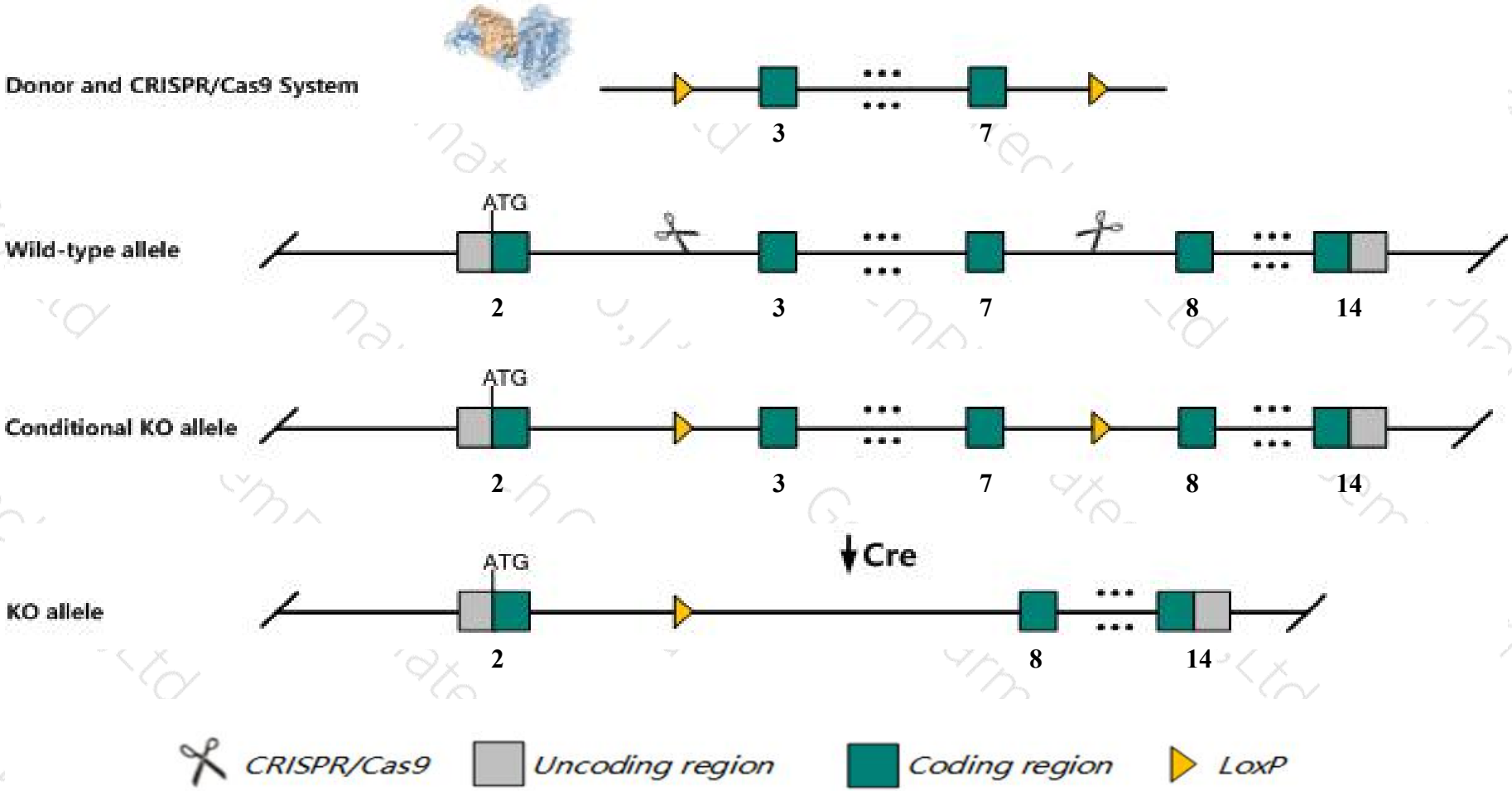
Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Lctl* gene has 6 transcripts. According to the structure of *Lctl* gene, exon3-exon7 of *Lctl-201*(ENSMUST00000034969.13) transcript is recommended as the knockout region.The region contains 587bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Lctl* 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,no gross notable phenotype was detected in knockout mice. Homozygous mice develop distorted eye lenses and cataracts, progressive with age.

➢ The *Lctl* gene is located on the Chr9. 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）

Lctl lactase-like [Mus musculus (house mouse)]

Gene ID: 235435, updated on 13-Mar-2020

Summary

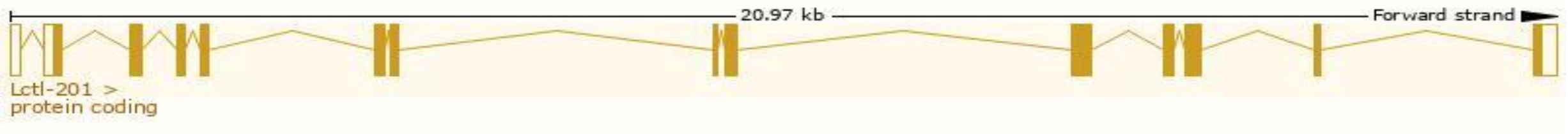
Official Symbol	Lctl provided by MGI
Official Full Name	lactase-like provided byMGI
Primary source	MGI:MGI:2183549
See related	Ensembl:ENSMUSG00000032401
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	E130104I05Rik, KLPH
Expression	Biased expression in subcutaneous fat pad adult (RPKM 18.1), liver E14 (RPKM 5.9) and 12 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

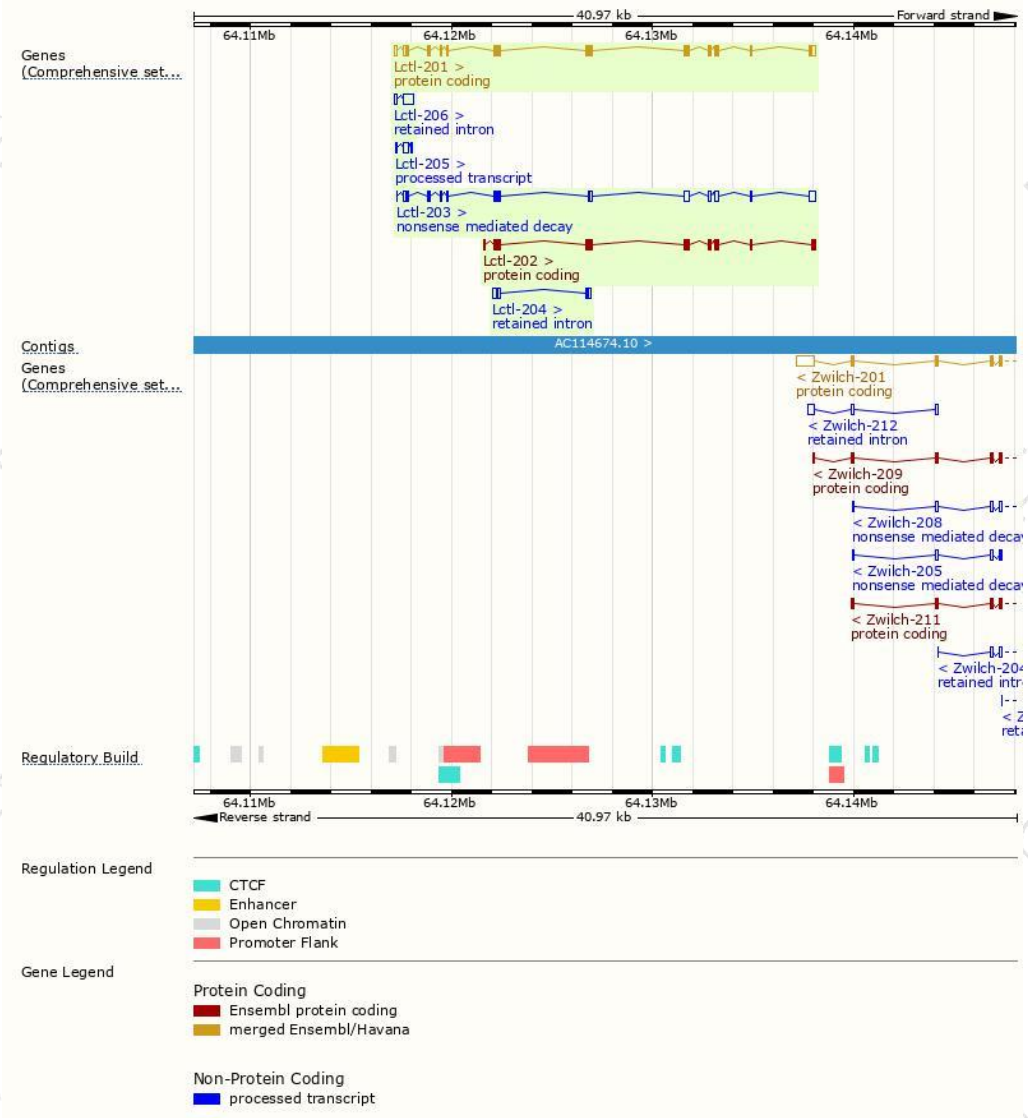
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lctl-201	ENSMUST00000034969.13	2171	566aa	Protein coding	CCDS23274	Q8K1F9	TSL:1 GENCODE basic APPRIS P1
Lctl-202	ENSMUST00000118215.2	1380	409aa	Protein coding	-	D3YTQ7	TSL:1 GENCODE basic
Lctl-203	ENSMUST00000124020.7	2000	235aa	Nonsense mediated decay	-	D6RI22	TSL:1
Lctl-205	ENSMUST00000139755.1	335	No protein	Processed transcript	-	-	TSL:3
Lctl-206	ENSMUST00000145011.1	650	No protein	Retained intron	-	-	TSL:2
Lctl-204	ENSMUST00000132018.1	440	No protein	Retained intron	-	-	TSL:3

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

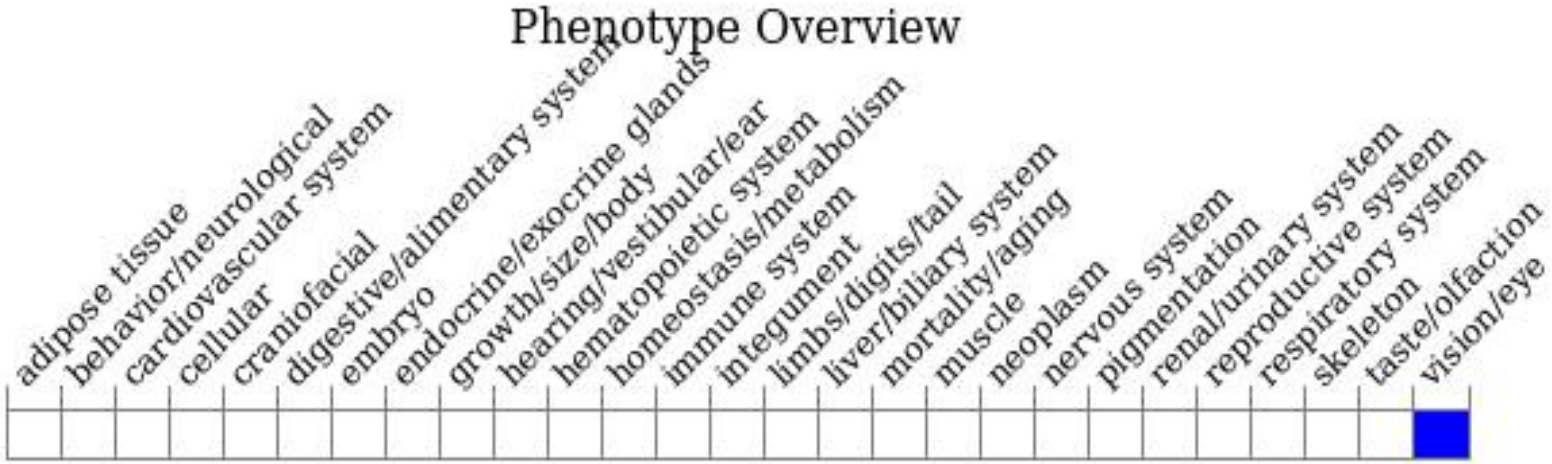


Genomic location distribution



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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