Clec1b Cas9-CKO *Strategy*

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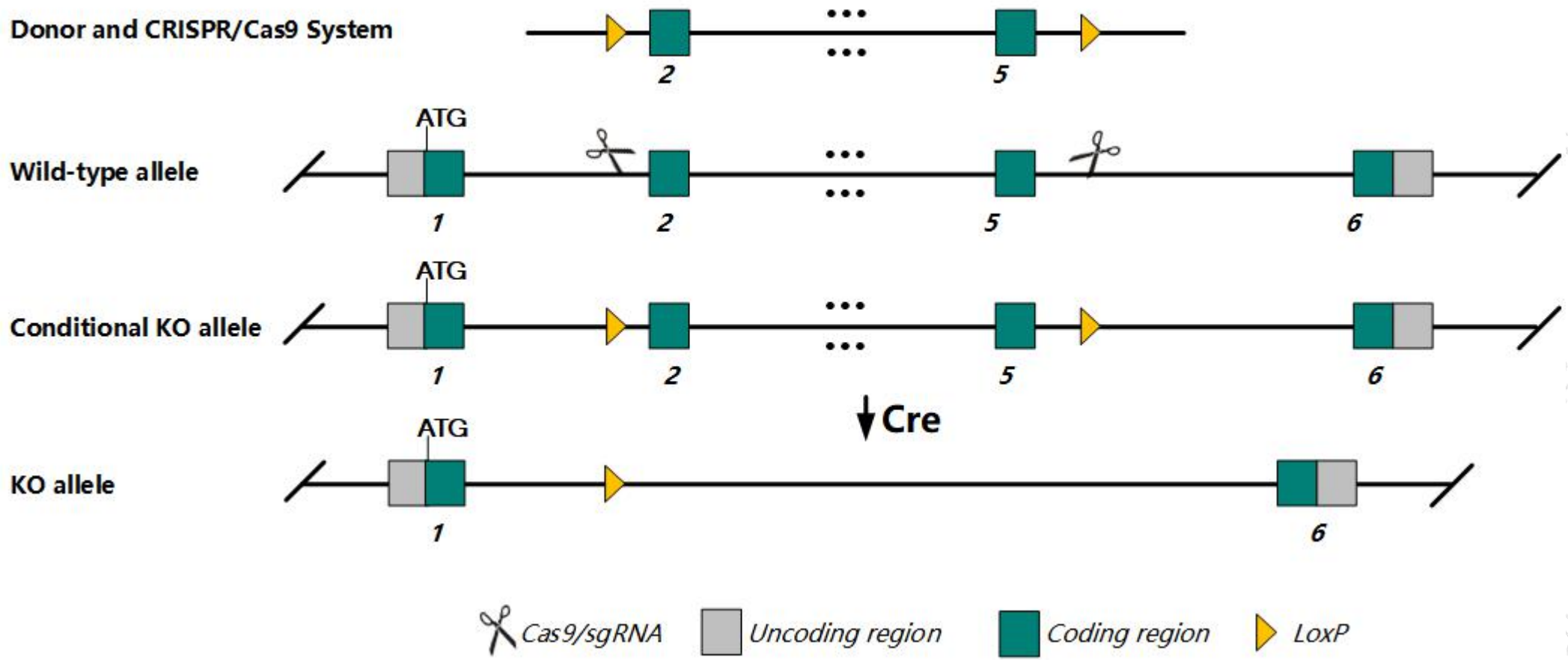
Project Overview

Project Name	***Clec1b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Clec1b* gene. The schematic diagram is as follows:



Technical routes

- The *Clec1b* gene has 4 transcript.According to the structure of *Clec1b* gene, exon2-5 of *Clec1b*-201 transcript is recommended as the knockout region.The region contains 481bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Clec1b* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 or cell types.

Notice

- According to the existing MGI data， Mice homozygous for a knock-out allele exhibit congestion and hemorrhages during embryogenesis with prenatal and postnatal lethality. Mice homozygous for another knock-out allele exhibit blood-lymph mixing, impaired PDPN-Fc-mediated platelet activation, and intestinal edema.
- The Clec1b gene is located on the Chr6. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

集萃药康 GemPharmatech

Pax6 paired box 6 [Mus musculus (house mouse)]

Gene ID: 18508, updated on 2-Apr-2019

Summary

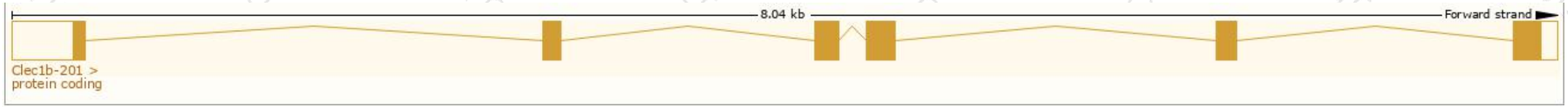
Official Symbol	Pax6 provided by MGI
Official Full Name	paired box 6 provided by MGI
Primary source	MGI:MGI:97490
See related	Ensembl:ENSMUSG00000027168
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1500038E17Rik, AEY11, Dey, Gsfaey11, Pax-6, Sey
Summary	This gene encodes a homeobox-containing protein that functions as a regulator of transcription. It plays a key role in the development of neural tissues, particularly the eye. Activity of this protein is also required for expression of glucagon in the pancreas. This gene is regulated by multiple enhancers located up to tens or hundreds of kilobases upstream and downstream of the transcription start sites. Mutations in this gene or deletion of these regulatory elements results in severe defects in eye development. Alternative splicing and the use of alternative promoters results in multiple transcript variants, some of which encode proteins that lack the N-terminal paired domain. [provided by RefSeq, Jul 2015]
Expression	Biased expression in cerebellum adult (RPKM 9.1), whole brain E14.5 (RPKM 7.7) and 6 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

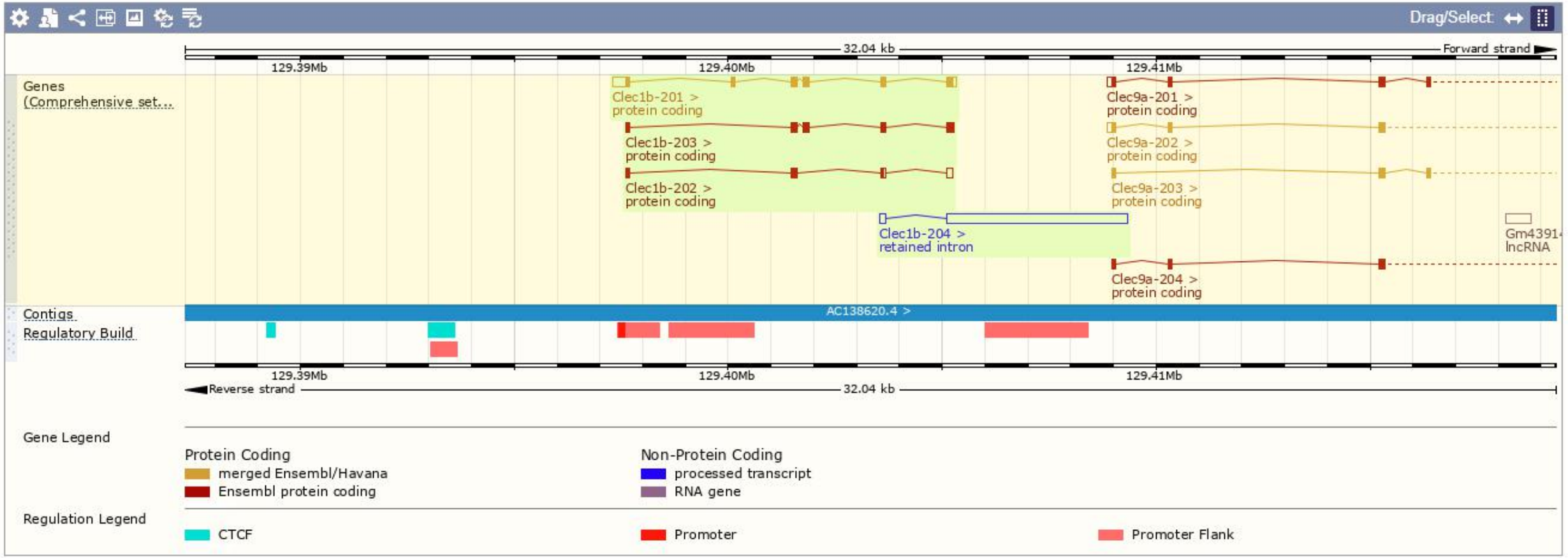
The gene has 4 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Clec1b-204	ENSMUST00000133061.1	4347	No protein	Retained intron	-	-	TSL:2
Clec1b-203	ENSMUST00000112081.8	614	197aa	Protein coding	CCDS57452	Q9JL99	TSL:1 GENCODE basic APPRIS ALT2
Clec1b-202	ENSMUST00000112079.2	455	83aa	Protein coding	CCDS57453	A0T1G3	TSL:1 GENCODE basic
Clec1b-201	ENSMUST00000032262.13	1090	229aa	Protein coding	CCDS20586	Q9JL99	TSL:1 GENCODE basic APPRIS P3

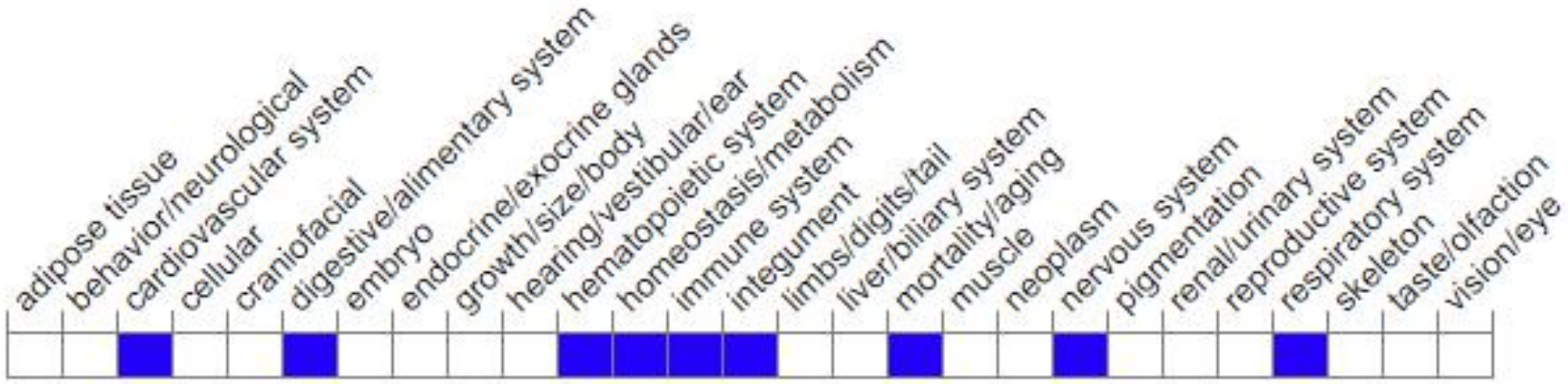
The strategy is based on the design of *Clec1b*-201 transcript,The transcription is shown below



Genomic location distribution



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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