

Cas2 Cas9-CKO Strategy

Designer:

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Design Date:

2019-7-19

Project Overview

Project Name

Cas^z1

Project type

Cas9-CKO

Strain background

C57BL/6JGpt

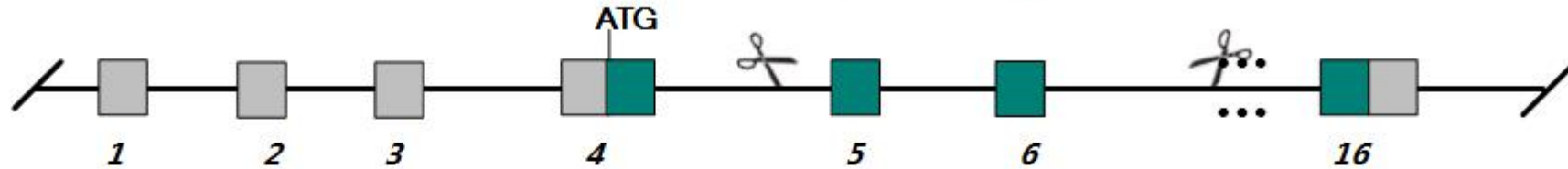
Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cas21* gene. The schematic diagram is as follows:

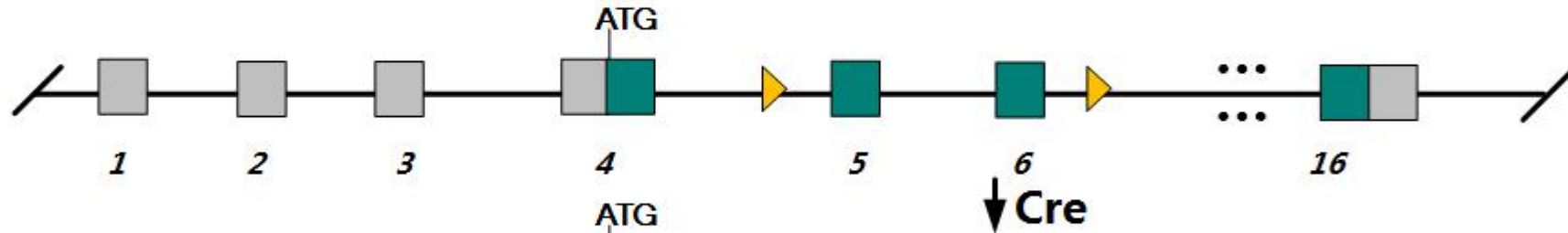
Donor and CRISPR/Cas9 System



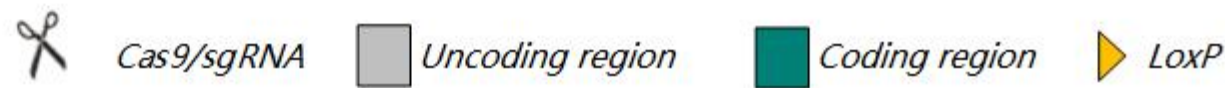
Wild-type allele



Conditional KO allele



KO allele



Technical routes

- The *Cas2l* gene has 6 transcripts. According to the structure of *Cas2l* gene, exon5-exon6 of *Cas2l-201* (ENSMUST00000094464.9) transcript is recommended as the knockout region. The region contains 1321bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cas2l* 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.

- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit complete lethality throughout fetal growth and development and abnormal heart development associated with edema, decreased fetal cardiomyocyte proliferation, myocardium hypoplasia, ventricular septal defect, and altered heart shape and Z line formation.
- The *Cas2l* gene is located on the Chr4. 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)

CasZ1 castor zinc finger 1 [Mus musculus (house mouse)]

Gene ID: 69743, updated on 3-Apr-2019

Summary



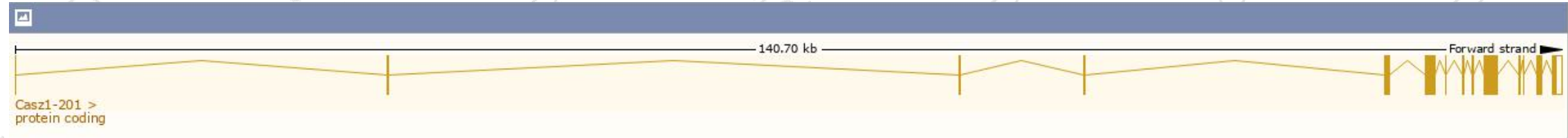
Official Symbol	CasZ1 provided by MGI
Official Full Name	castor zinc finger 1 provided by MGI
Primary source	MGI:MGI:1196251
See related	Ensembl:ENSMUSG00000028977
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2410019P08Rik, AV096627, BC040081, Cst, D4Ert432e
Expression	Broad expression in small intestine adult (RPKM 25.0), duodenum adult (RPKM 22.7) and 16 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

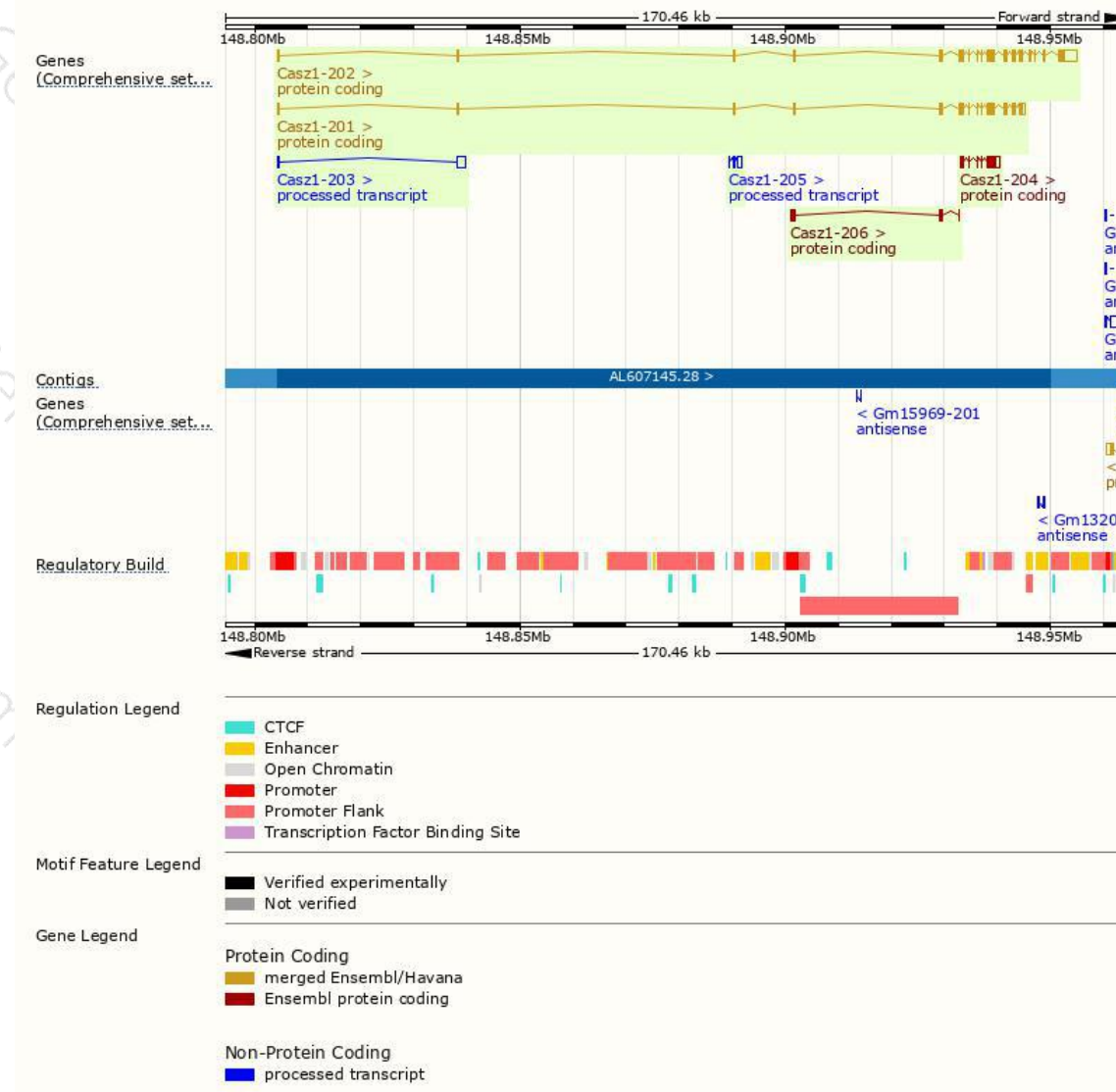
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
CasZ1-202	ENSMUST00000122222.7	7926	1762aa	Protein coding	CCDS51379	B1AS46	TSL:5 GENCODE basic APPRIS P1
CasZ1-201	ENSMUST00000094464.9	4349	1166aa	Protein coding	CCDS38973	Q9CWL2	TSL:1 GENCODE basic
CasZ1-204	ENSMUST00000139806.1	3014	702aa	Protein coding	-	B1AS48	CDS 5' incomplete TSL:1
CasZ1-206	ENSMUST00000147270.1	829	213aa	Protein coding	-	B1AS47	CDS 3' incomplete TSL:5
CasZ1-203	ENSMUST00000128881.1	1531	No protein	Processed transcript	-	-	TSL:1
CasZ1-205	ENSMUST00000140617.1	1140	No protein	Processed transcript	-	-	TSL:1

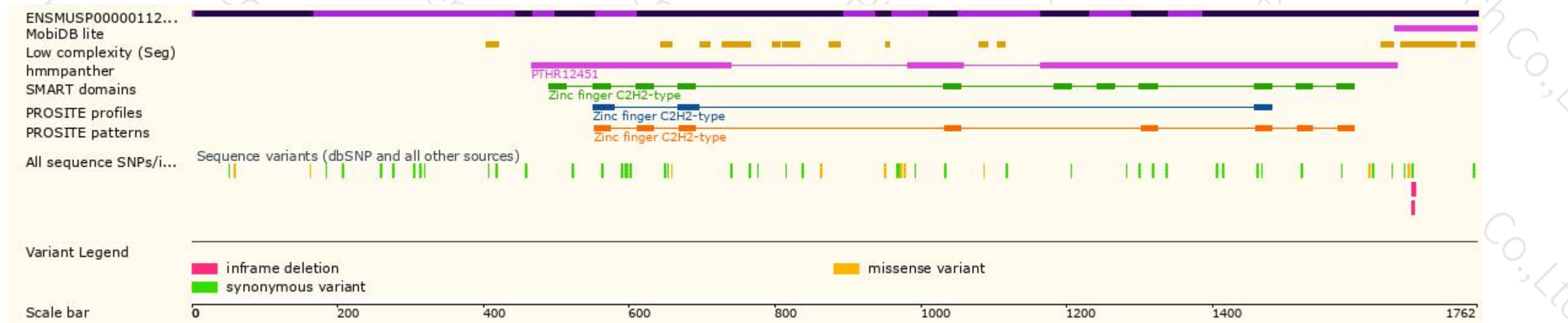
The strategy is based on the design of *CasZ1-201* transcript,The transcription is shown below



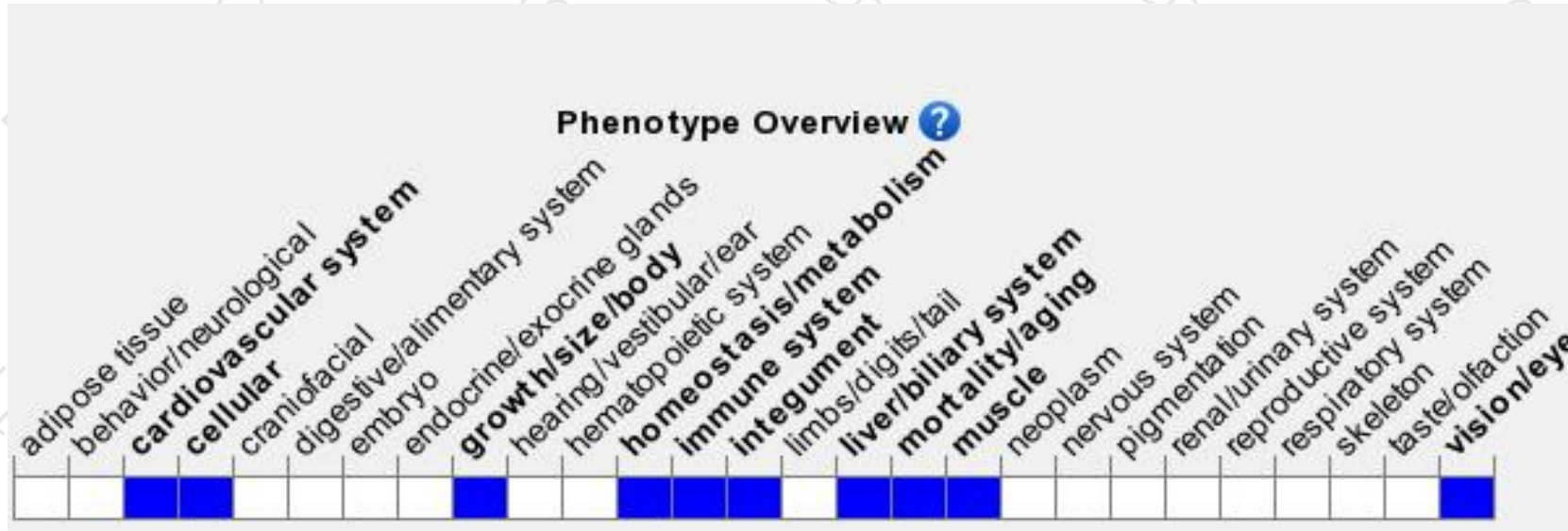
Genomic location distribution



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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