Bcl2l1 Cas9-KO Strategy

Designer: Huan Wang

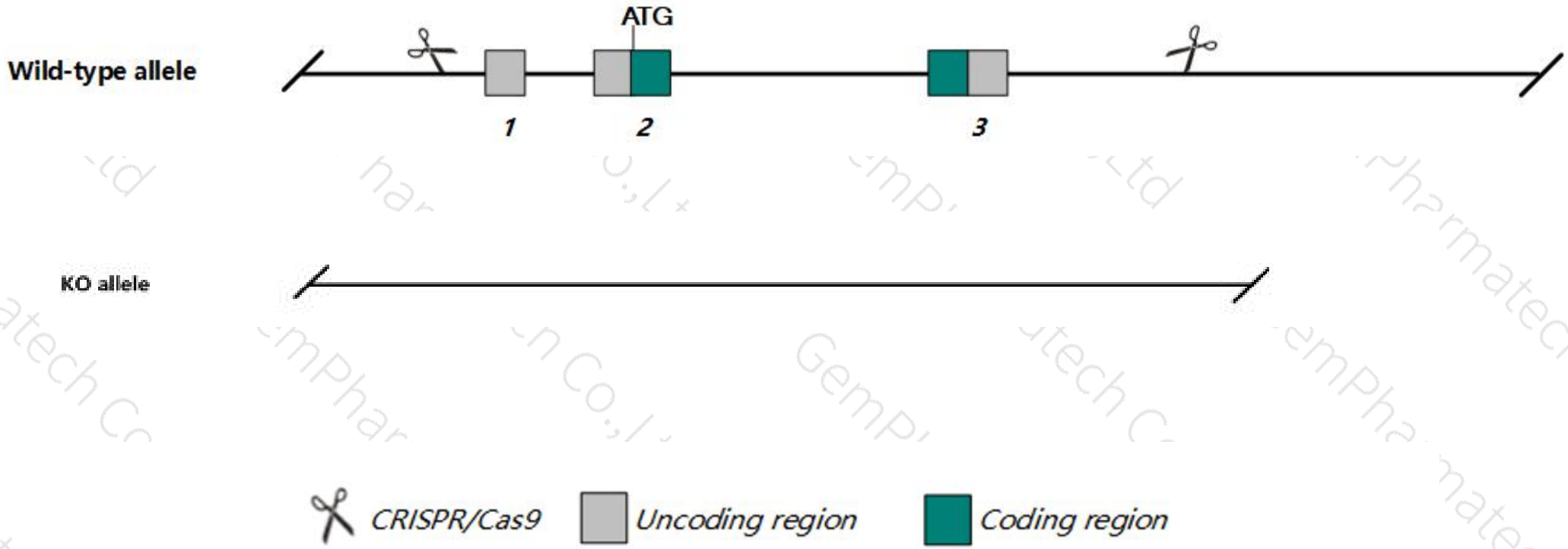
Design Date: 2019-7-22

Project Overview

Project Name	***Bcl2l1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Bcl2l1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Bcl2l1* gene has 9 transcripts. According to the structure of *Bcl2l1* gene, exon1-exon3 of *Bcl2l1-201* (ENSMUST00000007803.11) transcript is recommended as the knockout region.The region contains most of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Bcl2l1* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

Notice

➢ According to the existing MGI data,Homozygous null mutants die at embryonic day 13 with extensive apoptotic cell death, hypomorphic mutants have severe reproductive defects due to abnormal germ cell development. Mice lacking the gamma isoform show immune defects.

➢ The *Bcl2l1* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Bcl2l1 BCL2-like 1 [Mus musculus (house mouse)]

Gene ID: 12048, updated on 28-Mar-2019

Summary

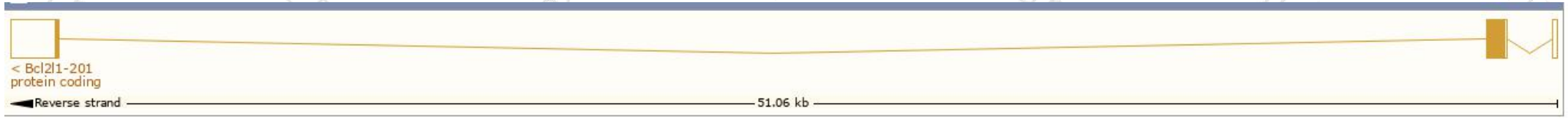
Official Symbol	Bcl2l1 provided by MGI
Official Full Name	BCL2-like 1 provided byMGI
Primary source	MGI:MGI:88139
See related	Ensembl:ENSMUSG00000007659
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Bcl(X)L, Bcl-XL, Bcl2l, BclX, bcl-x, bcl2-L-1
Summary	This gene encodes a member of the Bcl-2 family of apoptosis regulators. The encoded protein is localized to the inner and outer mitochondrial membranes and regulates the programmed cell death pathway during development and tissue homeostasis. This protein binds to voltage-dependent anion channels in the outer mitochondrial membrane to facilitate the uptake of calcium ions. Mice embryos lacking this gene survived for two weeks and exhibited cell death of immature hematopoietic cells and neurons. Alternative splicing results in multiple transcript variants. Additional alternatively spliced transcript variants of this gene have been described, but their full-length nature is not known. [provided by RefSeq, Jan 2014]
Expression	Ubiquitous expression in thymus adult (RPKM 18.5), liver E14.5 (RPKM 15.3) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

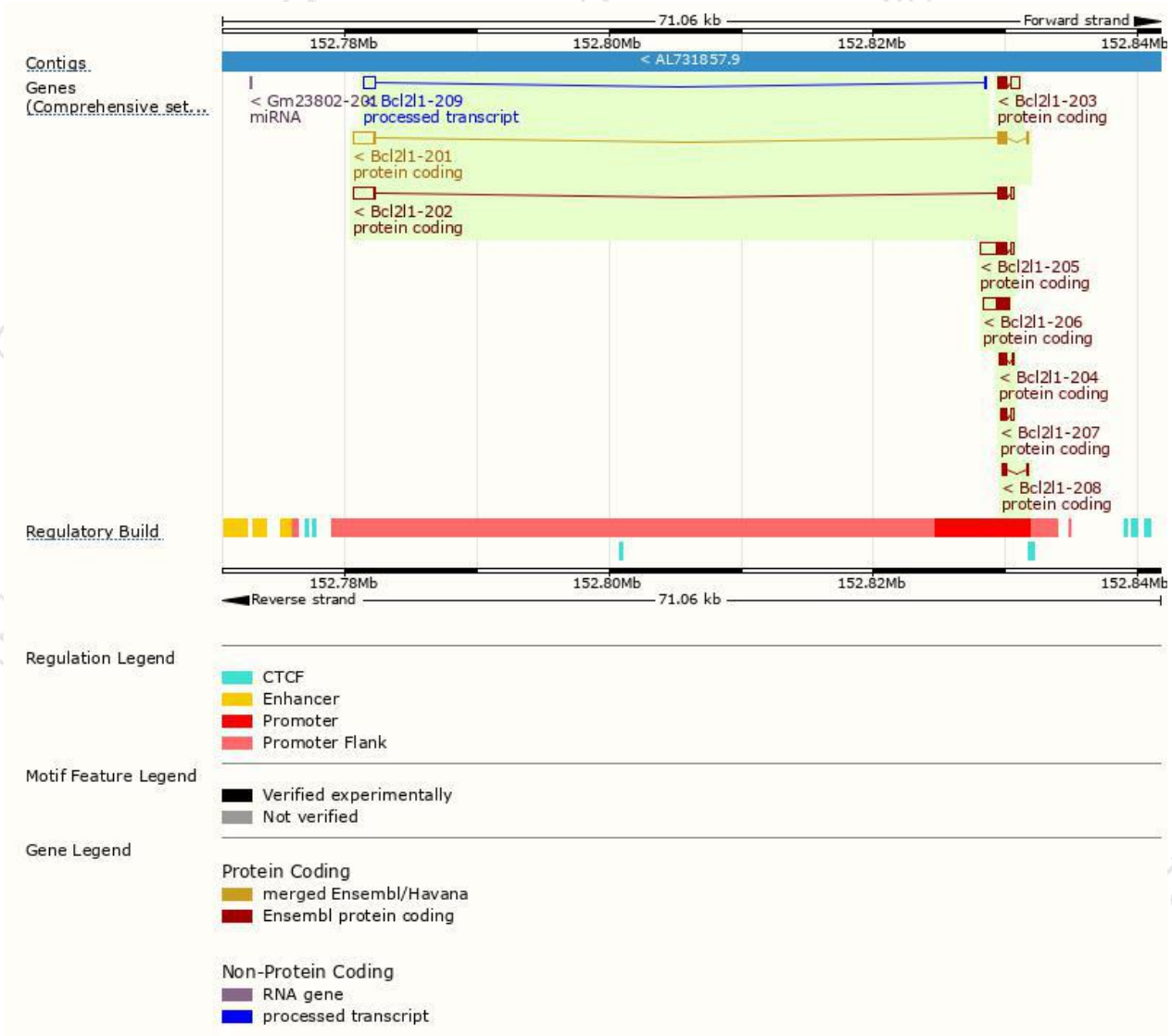
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Bcl2l1-209	ENSMUST00000173755.1	961	No protein	lncRNA	-	-	TSL:2
Bcl2l1-208	ENSMUST00000156688.1	472	78aa	Protein coding	-	A2AHX7	CDS 3' incomplete TSL:2
Bcl2l1-207	ENSMUST00000146380.1	653	96aa	Protein coding	-	A2AHX8	CDS 3' incomplete TSL:2
Bcl2l1-206	ENSMUST00000140436.1	1865	209aa	Protein coding	-	Q64373	TSL:2 GENCODE basic
Bcl2l1-205	ENSMUST00000134902.1	2232	209aa	Protein coding	-	Q64373	TSL:1
Bcl2l1-204	ENSMUST00000134357.1	686	154aa	Protein coding	-	A2AHX9	CDS 3' incomplete TSL:2
Bcl2l1-203	ENSMUST00000128172.2	1403	188aa	Protein coding	-	Q9QWX2	CDS 3' incomplete TSL:1
Bcl2l1-202	ENSMUST00000109820.4	2568	233aa	Protein coding	CCDS16899	Q5HZH3 Q64373	TSL:1 GENCODE basic APPRIS P1
Bcl2l1-201	ENSMUST00000007803.11	2417	233aa	Protein coding	CCDS16899	Q5HZH3 Q64373	TSL:1 GENCODE basic APPRIS P1

The strategy is based on the design of *Bcl2l1-201* transcript,The transcription is shown below

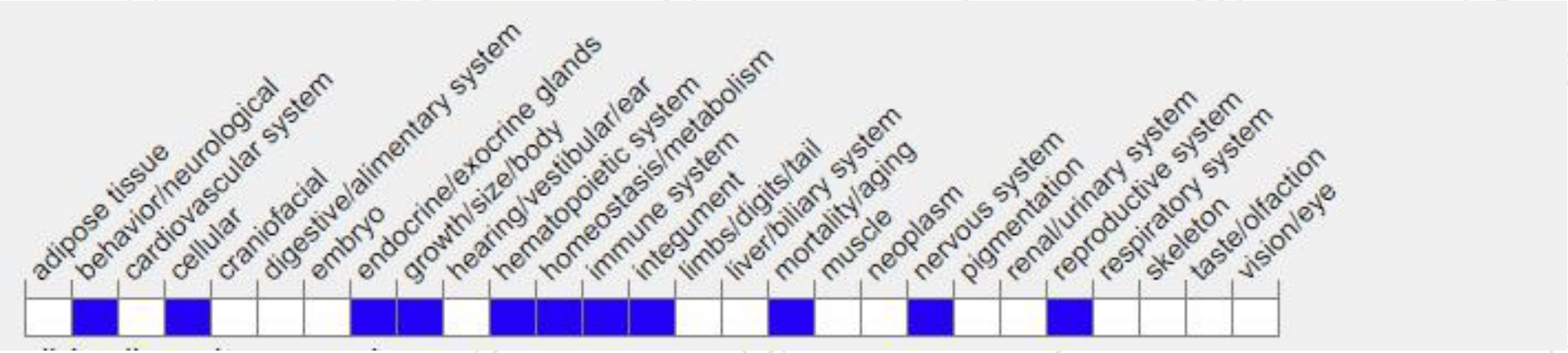


Genomic location distribution



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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